The influence of flushing diet composition on the reproductive performance of Dohne Merino (*Ovis aries*) ewes

by

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Declaration

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Chapter 1: General Introduction

South African agricultural activities that include forestry, hunting and fishing, contribute 2.4% to the total gross domestic product (GDP), which amounts to more than R270 000 million (Abstract of Agricultural Statistics, 2019). Of this R277 470,3 million GDP, more than 50% (i.e. R142 771.2 million) are derived from animal products (Abstract of Agricultural Statistics, 2019). However, in recent years there has been a decline in this contribution, which warrants investigation into approaches that will allow for the improvement of animal production to meet the food demand that is predicted for 2020 (Food and Agriculture Organization of the United Nations, 2019).

Livestock products in South Africa consists of wool, mohair, karakul pelts, ostrich feathers and products, fowls slaughtered, eggs, cattle and calves slaughtered, sheep and goats slaughtered, pigs slaughtered, fresh milk and other related products (Figure 1.1). Small stock (sheep and goats) contribute only R 12 867 159 000 000 of animal product income in South Africa. Meat makes up 60.6% of this income, wool 31.4%, mohair 7.9%, and karakul pelts only 0.2%. Most livestock income is derived from poultry (R 58 897 770 000 000), beef and veal (R37 318 286 000 000), and milk (R 17 814 543 000 000). Sheep and goats, with a gross turnover of almost R12.9 billion, do not contribute the highest income from the livestock sector (Abstract of Agricultural Statistics, 2019).

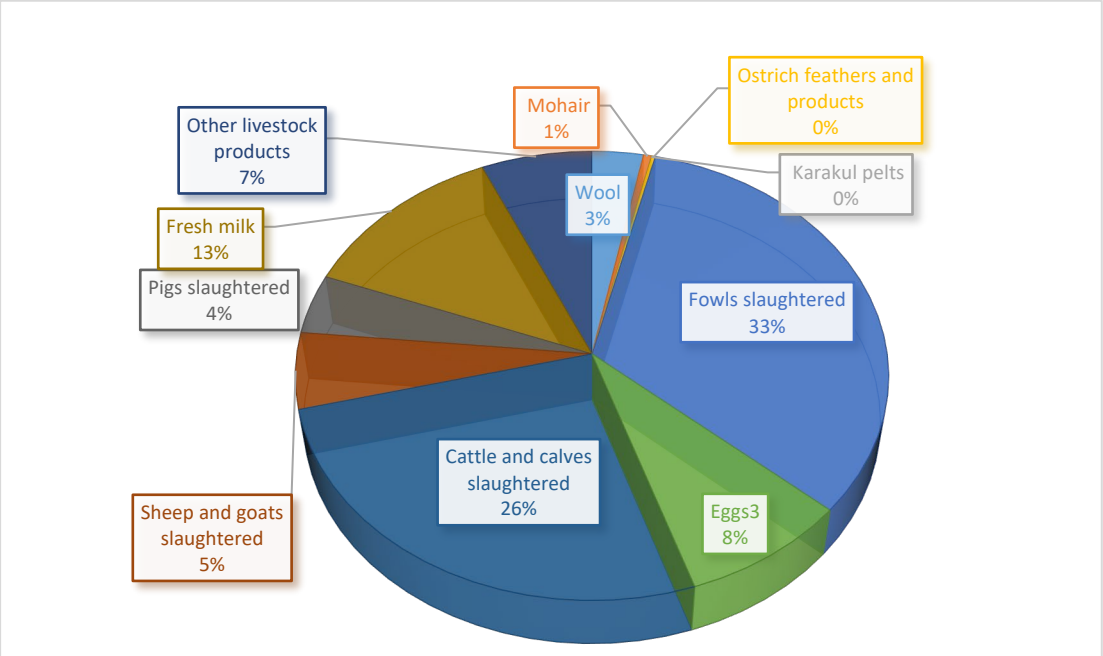

Figure 1.1. The percentage contribution of individual products to agricultural GDP in South Africa (Abstract of Agricultural Statistics, 2019).

Even though the involvement of sheep farming in South Africa seems little in terms of its financial contribution to GDP, the industry is of utmost importance to the economy when viewing from a regional context. This is especially important to consider when strategies are needed to allow for animal production to be carried out in the rural parts of the country (Cloete and Olivier, 2010). Of the approximately 86.2 million hectares of commercial agricultural land in South Africa, only 16.5% are arable. The arable land represents 14.2 million hectares that are suitable for crop production, with certain areas in the western and north-western parts of South Africa that are characterized by a grazing capacity of lower than 12 hectares per large stock unit. A large proportion of the arable land is used for large and small livestock farming, resulting in the relative income from livestock production being higher than it would have with either field crop or horticulture in these areas (AGIS, 2007b; 2007c).

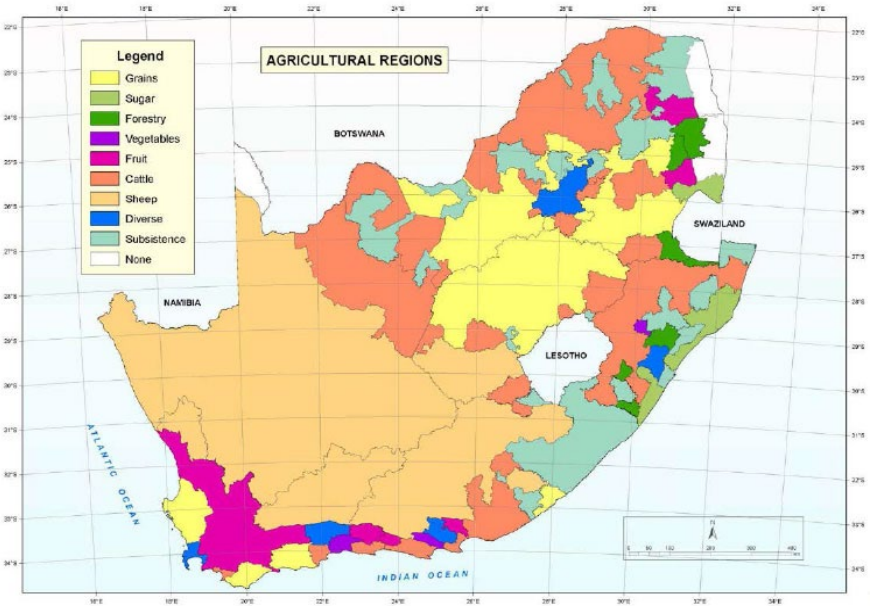

Figure 1.2. A map indicating the agricultural production activities per region in South Africa (Cloete and Olivier, 2012).

One of the most prominent income-generating industries in these areas is extensive small stock farming, with sheep production being the dominant enterprise in 20.5% of the Free State, 37.7% of the Western Cape, 50.8% of the Eastern Cape, and 82.0% of the Northern Cape (Figure 1.2; AGIS, 2007e).

The national sheep flock declined from over 28 million animals in the early 1980's to just more than 22 million in 2019 (Aginfo, 2011; Van Wyk, 2011; Cornelius, 2020). The diversification of domesticated livestock farming systems to game farming or more extensive rangeland livestock production systems had the most prominent impact on the decline in sheep numbers in South Africa and Namibia, with a consequent decrease in supply of sheep and related

products from these areas (Van Wyk, 2011). Very large areas in the southern parts of Africa is only suitable for extensive farming, and many livestock producers realized that game farming would be less risky in these areas and more productive. Game and cattle in these extensive farming systems are not as prone to stock theft as sheep, which presents another reason for the diversification and decline in sheep numbers (Vink and Kirsten, 2002; Cloete and Olivier, 2010). Aginfo (2011) stated that stock theft has a massive negative impact on the production of sheep products. The Red Meat Producers Organisation (RPO) of South Africa reported that 94 450 sheep were stolen from April 2011 to March 2012 (RPO, 2013). Predators such as black-backed jackal (*Canis mesomelas*) and caracal (*Caracal caracal*) contribute to stock losses, which in turn results in even less animals available for production purposes (Van Niekerk *et al.,* 2009; Dyson, 2013).

Total national meat consumption increased over the last 50 years, which can potentially be ascribed to an increase in the human population (DAFF, 2019). South Africa is considered a net importer of meat products, importing 42 000 tons of meat per year on average (Aginfo, 2011; Van Wyk, 2011). A change in prices of certain products is expected, if the demand is higher than supply for those products, according to conventional economic theory, which in turn will motivate producers to produce more to meet the demand (Pride and Farrell, 1993). Meat prices increased over the last ten years for all grades with more than 10%, despite the decline in the national sheep population in the last two decades (Aginfo, 2011; Van Wyk, 2011; DAFF, 2017). The consistent supply of meat to the national market are negatively affected by the decline in sheep numbers due to stock theft, and predators. In addition to stock theft and predators, feed availability and quality is also considered a limiting factor, especially in extensive sheep production systems. According to the Abstract of Agricultural Statistics (2019), the value of sheep products has increased from the years 2008 to 2018 (Figure 1.3).

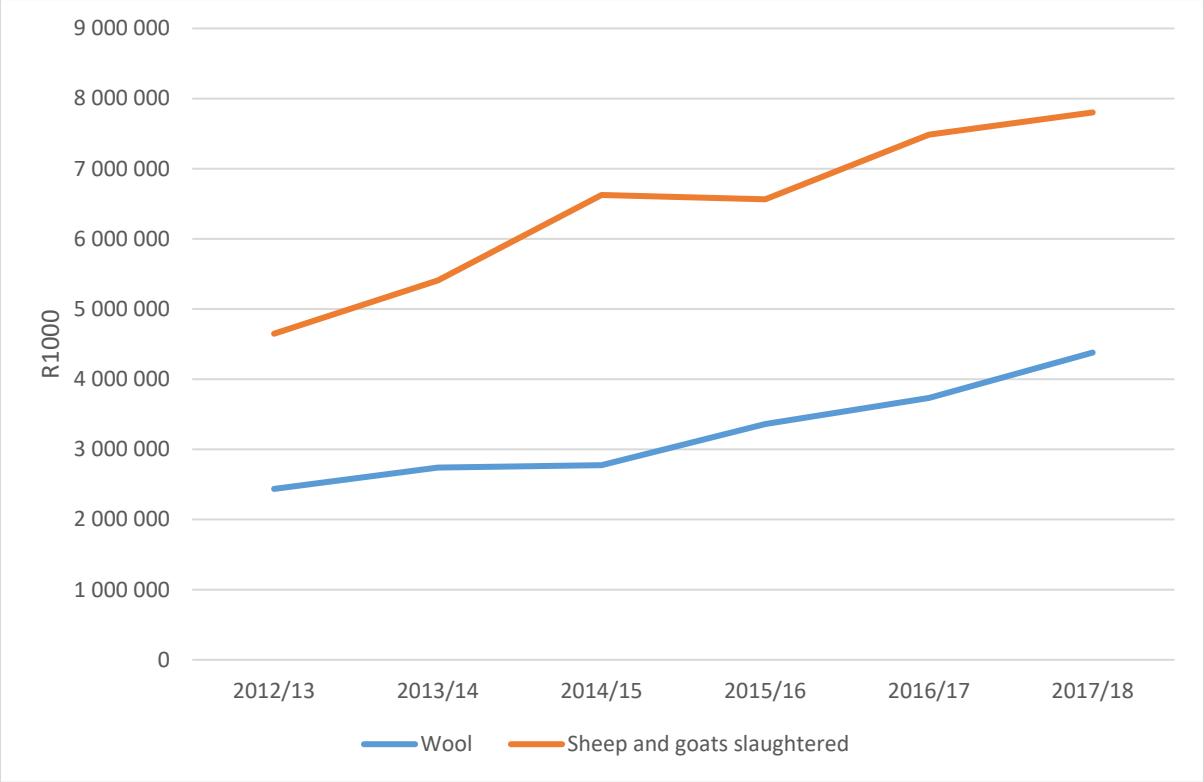

Figure 1.3. The increase in the value of small stock products from 2012 to 2018 (Abstract of Agricultural Statistics, 2019).

Taking this into consideration, it is clear that with the supply of sheep products from extensive rangeland production systems declining and in order to meet the expectancy of the conventional economic theory, sheep producers will strive to capitalize on the higher prices fetched for lamb by moving more towards intensive production systems. Bekker (2012) identified new possibilities regarding the production of sheep, using a more intensive approach by e.g. maintaining sheep on cultivated pastures to increase output per hectare.

Traditional pastoralism and extensive production for wool and meat are the two major farming management systems that are used worldwide for sheep production (Kilgour *et al.*, 2008). Production systems for sheep production vary from extensive systems (free range) to intensive controlled grazing (cultivated pastures, paddock rotations) or non-grazing feedlots, with all feed required for optimum growth provided in intensive systems. The choice of type of production system and management thereof, is determined by geography, available vegetation for grazing, and type of product produced (Maree and Casey, 1993). In sheep producing countries, extensive systems are most commonly used, and range from systems where sheep graze on large unfenced pastures and are micromanaged by shepherds, to systems where smaller flocks are kept in enclosed camps called lowland farming (Kilgour *et al.*, 2008). Maree and Casey (1993) indicate that the extensive grazing systems is mostly used

in areas of low rainfall. Xerophytic and succulent shrubs with mainly annual grasses are prominent in these areas. This kind of vegetation are palatable with high nutritional values with good resistance against high diurnal and seasonal temperatures. In South Africa is common practice in more moderate climatic areas to graze sheep and goats either separately or combination with cattle together, since each species grazes or forages a different spectrum of herbage. The lambing season of sheep in extensive production systems is highly dependent on the growing season of the natural veld to provide the ewes with enough reserves in terms of energy to raise their lambs. This season will also then be a determining factor in extensive system when to allocate rams to a mating flock.

When extensive sheep production systems are compared to more intensive approaches, productivity and profitability will be determined by the viability, sustainability, and optimal management of these systems. In South Africa, it is common practice for extensive systems to produce a minimum of one offspring/ewe/lambing season that can be marketed, which is also dependant on the seasonality and patterns of pasture or veld growth (Nel, 1980; de Nicolo, 2007). In intensive sheep production systems, the focus is on an increase in the stocking rate by increasing the quality and quantity of feed provided to the animals, e.g. using cultivated pastures or full ration feeds to increase the total output per unit (Bouwman, 2007).

Standard industry practice in South Africa is to allocate rams for a period of six weeks per year, with an inclusion rate of 3% to 4% rams to a mating flock of 100 ewes (Brand and de Villiers, 1989). The joining ratio and time frame are based on an estimation of the time and frequency of exposure of ewes to enable successful conception. An oestrus cycle of an ewe is 17 days in duration, and an ewe potentially has 2.5 opportunities in the 42-day period to conceive. Most farmers in South Africa select against and cull ewes that do not conceive in a breeding period of 60 days in order to increase conception rates and lambing percentage of the national breeding population (Gootwine, 2016).

The importance of the nutrition of the ewe, especially in the time prior to the introduction of the ram, should not be underestimated. Flush feeding entails the feeding of a diet higher in energy and/or protein for a minimum of two weeks prior to and two weeks after the introduction of the ram (Habibizad, Riasi, Kohram and Rahmani, 2015). The increase in available nutrients and thus better body condition, in combination with the use of teaser rams, increase the conception potential of ewes. In contrast to extensive systems, intensive systems make use of cultivated pastures and/or feedlots, where the feeding of rams and ewes are controlled to maximise the carrying capacity per hectare (Maree and Casey, 1993). Some intensive sheep production systems manipulate reproduction through oestrus cycle synchronisation, which allows do a

more direct control of production and reproduction potential of the flock. The aim with this type of intensification is to have ewes lamb more than once a year, and mostly in lambing pens to accommodate the ewes with multiples better. Production and profitability per hectare potentially increase with intensification, but is accompanied with an increased workload, labour required, costs, and risks. Nutrition of breeding animals is considered as a high-risk factor, for almost 70% of input costs consists of feeding the breeding population (Kilgour *et al.*, 2008). Poorly planned and managed feeding regimes can result in overweight animals, which in turn will impact negatively on the fecundity of a flock (Velazquez, 2015).

Nutrition is widely acknowledged to interact with the seasonality and cyclicity of reproduction in sheep, likely through the central hypothalamic pathway. Nutritional status of the ewe, before or after ovulation, may affect oocyte development, embryo development, and successful establishment of pregnancy (Sen *et al.*, 2013; de Brun *et al.*, 2016). Nutrition is considered as one of the most important factors influencing the reproductive efficiency of ewes, with improved nutrition that can exert short- and long term effects on ovulation rate, lambing rate and litter size (Montgomery *et al.*, 1988; Lassoued *et al.*, 2003; Abdel-Mageed and Abd El-Gawad, 2015). The use of a short-term flushing period can increase follicle development and advance the time at which ovulation will occur. Ocak *et al.* (2006) found that short-term supplementation (i.e. 15-17 days) post-mating can beneficially influence the non-return rate and lambing rate after the first oestrus, as well as litter size.

It is important for sheep producers, regardless of production system, to have access to diets specifically formulated to optimize the reproductive ability of their ewes. When the composition of the flushing diet is considered, it is important that supplementation with a flushing diet results in a significant weight gain in ewes that will impact positively on the ovulation and conception rates, and ultimately lamb weaning success (Friedman and Turner, 1939; Clark, 1934; Darlow and Hawkins, 1933; Marshall and Potts, 1924). Several studies stated that fodder of good quality, whether it be pastures of grain, and that is considered palatable by the animal, will have the desired effect in ewes receiving such supplemental flush feeding (Geary, 1956; Bray, 1925). Several studies have also reported that the inclusion of lupin grain in a supplement ration before mating can positively influence the reproduction rate in sheep.

Including fatty acids in the supplementary ration can impact ovarian function and follicular development positively and in combination with the energy provided, enhance the reproductive performance in ruminants (Gulliver et al., 2012a; Nieto et al., 2015). The variety of fatty acids available in different lipids can impact the animal's performance and reproductive functions differently. Polyunsaturated fatty acids (PUFAs) are lipids containing 16- 22 carbon

atoms with 2 or more double bonds in the carbon chain. Feeding dietary omega-6 (n-6) or omega-3 (n-3) PUFA has positively impacted the reproductive success in sheep and cattle (Gulliver et al., 2012a). Increased number and size of pre-ovulatory follicles (Nieto *et al.*, 2015), improved conception rates, improved embryo quality and calving rates resulted from feeding lactating dairy cows various sources of PUFAs.

Long-chain polyunsaturated fatty acids (PUFA) like n-3 eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and n-6 arachidonic acid (AA, 20:4n-3) are synthesised in the body from the short- chain n-3 linolenic acid (ALA, 18:3n-3) and n-6 linoleic acid (LA, C18:2n-6) through desaturation and elongation. The short-chain ALA and LA cannot be synthesised by animals and are thus considered as essential and, therefore need to be supplemented in the diet (Lands, 1992). There are several sources of the short-chain n-3 ALA in ruminant diets, including certain forages and linseed. Long chain n-3 (20 carbons or more) purified from sources such as fish oil and fishmeal can also be fed to ruminants and these are usually rumen undegradable (Ashes *et al.*, 1992).

These supplements are usually expensive, however it may potentially accumulate in higher profits in animal yield. Given the background of the contribution of specific raw materials to these rations and due to the competitiveness of the feed industry, it is important to validate the potential of such diets to enhance or improve follicle development, ovulation rate, embryo survival and ultimately weaning success. It is therefore necessary to determine the effect of flush feed diet composition on ewe conception rate, number of lambs born and lamb birth weight, to determine the financial implication of such diets for use in extensive and intensive sheep production systems.

In the South African animal feed industry, certain animal feed companies formulate diets that are referred to as “super-fertility” diets, which are considerably more expensive than standard flushing diets. The assumption that the feeding of such “super-fertility” diets will result in an improvement of weaning percentage of flocks through having a beneficial effect on follicular development and subsequent conception rates of ewes, which in turn will decrease input costs for the sheep producer. No studies have however been conducted to verify these assumptions. The aim of the study is therefore to determine and validate the potential of a commercially available fertility diet (also referred to as a super-fertility diet) to enhance follicle development, and number of lambs weaned per ewe joined, as well as to assess the economic implications of the use of such diets in sheep production systems.

Chapter 2: Literature review

2.1 The South African sheep industry

The agricultural sector plays an important role in South Africa, with a total of 16.5 million people that are employed in the agriculture, hunting, forestry, and fishing sectors of the country (Abstract of Agricultural Statistics, 2019). In the years 2017/18, animal production generated a gross income of R142 771.2 million in South Africa, and of that, more than R4.6 million of this income was generated from sheep and goats slaughtered, and more than R2.4 million from wool, respectively (Abstract of Agricultural Statistics, 2019).

In South Africa, the various sheep breeds farmed with are classified according to the product produced, resulting in the classification of either wool, lamb/mutton, and dual-purpose sheep breeds. The choice of breed that is farmed with is determined by the geographical location, and thus suitability of a particular environment for that breed, to allow for the maintenance of animal wellbeing, and also to ensure viable, cost-efficient and sustainable production. Dual-purpose breeds originated from a need to meet the demand for mutton and wool, and to overcome the slow growth rate of lambs of wool breeds. Examples of dual-purpose breeds farmed with in South Africa include the Afrino and Dohne Merino.

In South Africa, commercial and communal sheep farming predominantly occurs in the western, southern, and central areas of South Africa (Cloete and Olivier, 2010). The Eastern Cape are known for having the most communally farmed sheep. The Northern Cape, Free State, Eastern Cape, and Western Cape are the provinces with the most commercial sheep producers in South Africa, and the largest sheep population can be found in the Northern Cape (Table 2.1). Table 2.1 presents the distribution of sheep per province throughout South Africa.

Table 2.1. The provincial distribution of the South African sheep population that occur in communal and commercial production systems (adapted from Cloete and Olivier, 2010).

Province	Sheep numbers (X 1000)		
	Commercial	Communal	Total
Western Cape	2 881	20	22 881
Northern Cape	7 811	79	86 811
Free State	5 862	169	174 862
Eastern Cape	5 658	2967	2 972 658
KwaZulu-Natal	700	158	858
Mpumalanga	1 645	18	19 645
Limpopo	91	150	241
Gauteng	82	0	82
North West	525	209	734

The initial primary purpose of sheep production was to produce wool, with mutton as an additional income consequence. However, around 2000 and due to a decline in wool prices, mutton became the main income generating product when wool-sheep flocks are considered (Hoon *et al.*, 2000). Currently, almost 60% of the income generated from the national small stock population is derived from meat products. Despite the local demand for meat products, the value of lamb and mutton imported by South Africa is higher than the value of meat exported, and South African can thus be considered a net importer of meat (Abstract of Agricultural Statistics, 2019).

According to the Abstract of Agricultural Statistics (2019) and as depicted in Figure 2.1, the size of the national wool sheep population (i.e. including Merino and dual-purpose breeds) decreased from 25.0 to 14.1 million from 1970 to 2018. The number of meat sheep (i.e. mainly the Dorper breed) increased from 3.7 to 7.8 million from 1970 to 1999, and then declined to an estimated 5.5 million sheep in 2018.

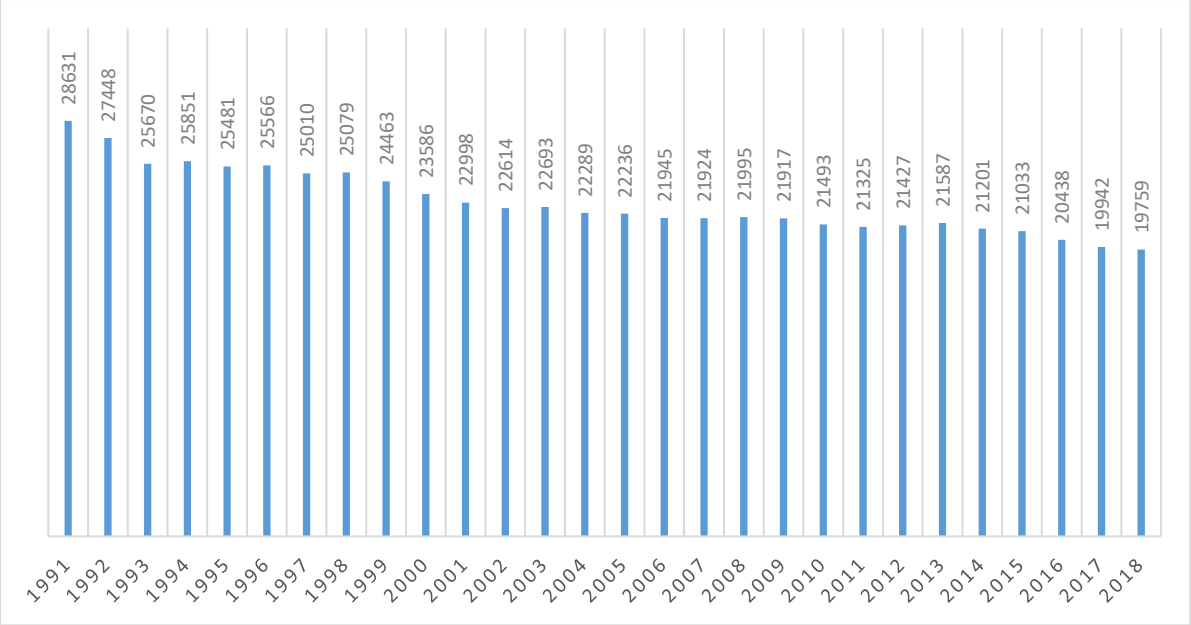

Figure 2.1. The fluctuation in the size of the South African sheep population in the period from 1991 to 2018 (adapted from DAFF, 2019).

The diversification of domesticated livestock farming to game farming or more extensive rangeland livestock production systems, contributed significantly to the decline in sheep numbers in South Africa and Namibia (Van Wyk, 2011). Game and cattle in extensive farming systems are not as prone to stock theft as sheep, which contributed to the decision of sheep farmers to convert to game/cattle production (Vink and Kirsten, 2002; Cloete and Olivier, 2010).

As a result of a health-conscious revolution worldwide, the per capita consumption of red meat declined, however the demand for sheep products in South Africa has increased (Fiems,1987). This contradicting statistic can potentially be ascribed to the rapid population growth in South Africa (Morokolo, 2011). This increase in demand and decline in sheep numbers thus result in a shortage, which in turn increased the value of mutton (Morris, 2009). In Figure 2.2, a constant increase in the average price for mutton can be seen from 1991 to 2019 (adapted from DAFF, 2019). This trend of increased price of mutton may continue given the decline in sheep numbers nationally and internationally. Conventional economic theory suggests that if demand is higher than supply, a change in price levels can be expected, which in turn provides an incentive for producers to increase supply (Pride and Ferrell, 1993).

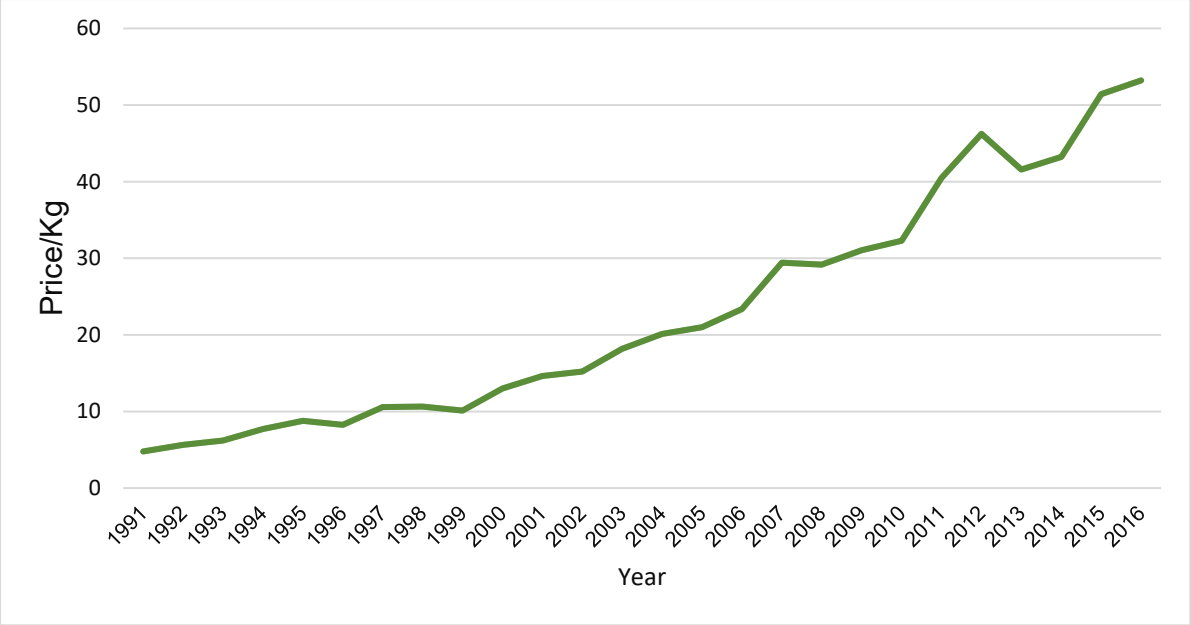

Figure 2.2. The annual change in mutton prices in South Africa in the period 1991 to 2017 (adapted from DAFF, 2019).

2.2 Sheep production systems

Sheep are farmed on both an intensive and extensive scale in South Africa, with the choice of production system determined by the suitability of the breed for a particular production environment. Each type of production system is characterized by advantages and disadvantages, which will be discussed below.

2.2.1 Extensive production systems

Sheep farming allows sustainable production in extensive pastoral areas where no alternative farming ventures can be practiced, such as the vast extensive Karoo regions of the central part of South Africa. The dry western and central districts have sheep production as the dominant industry, with sheep production being the dominant enterprise in 20.5% of the total area of the Free State, 37.7% of Western Cape, 50.8% of Eastern Cape and 82.0% of Northern Cape (AGIS, 2007e). Extensive farming systems involve the use of a limited number of personnel on the farm, as well as minimal capital input in relation to the area of land being farmed on. To keep input costs low, it is important to use breeds suitable for an extensive production environment (Nel, 1980). Input costs for extensive sheep production are mostly determined by management practices, type of facilities available or used, and management of the breeding of sheep under extensive conditions.

Extensive rangeland conditions are known for having lower carrying capacities (Cloete and Olivier, 2010). In extensive sheep production systems rearing body weights, reproduction

rates and milk production are generally lower, and mortality rates are higher due to the poor nutritional quality of the vegetation the sheep have access to. In South Africa, it is common for producers with extensive systems to produce at least one marketable lamb per ewe per year. This aim is obviously limited by the seasonal pattern of veld growth in these areas, which in turn will influence the fecundity of ewes in such systems (Nel, 1980; de Nicolo, 2007). In extensive systems, the lambing season of sheep is predominantly determined by the growing season of the natural veld in a specific area. The aim is to provide the ewes with the best veld conditions and thus potential to raise their lambs on natural veld, without any supplementary feeding provided to either the ewe or the lambs, in an effort to reduce input costs (Nel, 1980).

In most extensive production systems, rams are allocated to ewes for a period of 42 days, at a ratio of one ram for every 100 ewes (Brand and de Villiers, 1989). Due to the seasonal nature of reproduction in the ram and ewe in temperate regions, the ram is introduced only once a year in extensive mating systems. Bearing in mind that an ewe's oestrus cycle is approximately 17 days in duration, this results that in the 42-day period ewes have on average 2.5 opportunities to conceive. In extensive systems, the practice is to cull ewes that do not conceive during a 60-day period (i.e. in case a ewe conceive on the last day of the 42-day period), which assists with the selection for higher conception rates and overall lambing percentages in a breeding flock.

A widely used practice in terms of supplementary feeding is flushing. Female sheep gaining weight or fat reserves in the 3-4 weeks before mating, is more likely to conceive and have twins or triplets than those in poorer condition. This has led to the practice of "flushing" ewes by transferring them from a low to a high plane of nutrition before mating. In extensive systems, other supplementations are usually required to overcome certain mineral deficiencies commonly known to certain production areas. These deficiencies are usually soil deficiencies and the supplementation of the sheep will differ from location to location.

In extensive production systems in South Africa common practice is to wean lambs at 120-150 days of age. This weaning age will differ between breeds and the lambs are either placed in feedlots to finish them off or on different pastures. Lambs are separated from the ewes so the ewes can re-enter the reproductive cycle to generate more revenue by producing more lamb. For a most ideal situation, the weaned lambs reach marketable weights on the veld and can be marketed directly off the veld with minimal input cost.

Extensive systems seem to require less labour with low risk, and if a farmer has the land, an extensive production approach seems a good choice. However, the vulnerability of sheep to theft and predators is of utmost concern. Aginfo (2011) reported stock theft has a massive

negative impact on the supply of sheep products, and the Red Meat Producers Organisation of South Africa (RPO) estimated that 94 450 sheep were stolen from April 2011 to March 2012 (RPO, 2013).

Extensive systems are also characterized, when compared to semi-intensive and intensive sheep production systems, by major stock losses as a result of predation by predators such as black-backed jackal (*Canis mesomelas*) and caracal (*Caracal caracal*). Predation control is complicated by the fact that these two predators are now protected species, and the use of anti-predator measures to control predator numbers is legally not permitted. In 2007, the Red Meat Producers' Organisation stated that the national predator situation is a bigger problem than animal theft. It is confirmed that farmers lose up to 8% (2.8 million in numbers) of their small livestock to predators (Van Niekerk *et al.*, 2009). According to Gerhard Schutte, Chief Director of the RPO, these losses associated with predation amounts to R1.4 milliard per year (Van Niekerk, 2010), which is four times as much as the loss of stock theft (Botha, 2009).

2.2.2 Intensive production systems

Intensive sheep production systems are becoming increasingly more common in South Africa due to the increased demand for sheep products, and because producers are changing from extensive production systems to systems where they have more control and thus is in a better position to overcome challenges of e.g. predation.

Intensification of sheep production implies that the output obtained per production unit needs to be increased. Typically, the output of a system can be increased by increasing the carrying capacities per hectare, which is made possible by the introduction of cultivated pastures or by placing animals in feedlots where the animals are fed daily. These feeding strategies, in combination with improved breeding strategies are used to optimise lambing percentage.

With regards to intensive production practices, the measure of intensity is most clearly visible in terms of management (Bekker, 2012). Some common intensive management practises will include cross-breeding within the flock to reassure robustness, seeing that production of lambs is the main measured output, with permanent flocks consisting of breeding rams, ewes and also replacement ewes. In common intensive systems grazing pastures are cut and baled in seasons when there is a surplus, and used in other seasons when there is a shortage of feed or when the phase of production demands higher input. Breeding ewes will be removed from the veld during late pregnancy, and maintained in feedlots where they will lamb and wean their offspring. From there, the ewes will be serviced again and put back on the veld. In some practices there is no permanent flock. Low value pregnant ewes are bought and fed in feedlots

or on pasture. These ewes and their lambs will then be marketed after weaning. This process thus can merely repeat itself. Other intensive production systems take on the risk of dry seasons, where weaned lambs are bought and fed to finish. This higher risk of the so-called “dry season” approach, is because of the inconsistent availability of weaned lambs.

Nutrition of the breeding animals, as well as quality of diet components, are the most crucial aspects of intensive sheep production. It is commonly accepted by farmers that the higher the level of intensity of the production system, the more attention to detail are required in terms of nutrition (Owens *et al.*, 1993). Nutrition is the aspect of the production system that the producer can control to a large extent, i.e. when and what to feed (Chappell, 1993). Whether the producer relies on cultivated grazing pastures or feed for feedlot systems, each system will have unique challenges and requirements in terms of nutrition.

Coetzee (2010) stated that planning can make or break being profitable. He realized that less profitable producers lack the ability to adjust management and/or set poor targets. The more profitable producers evaluate their determined targets more frequently and adjusts management accordingly. Returns on investment of extensive farming are much lower than intensive farming, but the more intensive in nature a system is, the higher the risks become, and more management input is required (Roeder, 2007a). Wessels (2011) confirms this by stating that higher labour and capital inputs are required for intensive sheep farming.

Important factors to consider when diversifying to intensive systems include amongst others, initial capital, water and electricity consumption and costs, and the work force required to manage the sheep population (Bezuidenhout, 1987; Breytenbach *et al.*, 1996). Du Plessis (undated) stresses the importance of accurate economic analyses and planning before any farming enterprise is considered. He also agrees with the aforementioned facts that the investment needed to establish intensive production systems is capital demanding, therefore to achieve financial success, ‘above average’ level of management is required (Landman, 2013).

2.3 Sheep as a production animal

Sheep breeds are adapted to thrive in arid environmental conditions. Sheep are very agile and graze easily on rugged mountain terrains, where cattle choose not to feed. Some sheep breeds is also well adapted to survive on sparse desert range that would not be used otherwise. Given that most of South Africa’s surface is characterized as non-arable with a low rainfall, the physiological adaptations of sheep allow them to cope better with environmental conditions that may e.g. result in a degree of dehydration (Mirkena *et al.*, 2010; Cloete and

Olivier, 2012). Sheep this is considered to be the most effective livestock species in South Africa.

The age at which a ewe reach puberty is influenced by breed, genetics, live weight, nutrition status, and season of breeding (Smith and Clarke, 2010). Most ewe lambs reach puberty between 5 and 12 months of age. A ewe is pregnant for 142 to 152 days, approximately five months or slightly shorter (Fitzgerald *et al.*, 2015). The live weight of new-born lambs can be influenced by breed, sex of lamb, litter size, and ewe nutrition. The lambs from medium to small breeds are similar in size to human babies, with an average of 3.5kg to 4.5kg (Gardner *et al.*, 2007). In general, natural selection pressure has favoured the propagation of those genes that link the time of birth to the most appropriate phase of an annual cycle of food availability, i.e. in early spring (Jewell *et al.*, 1974; Ortavant *et al.*, 1985; Short, 1985).

The most important factors influencing lamb growth rates pre-weaning are ewe milk production from lambing to weaning (which is influenced by pasture available to ewes, ewe condition at lambing, and ewe genetics), and pasture quality during late lactation. The "best" time to wean is determined by various factors such as facilities, availability of pasture and other feed supplements, and target markets. Lambs have been weaned successfully as early as 14 days (rare and not recommended), while some lambs are allowed to wean naturally, staying with their dams for six months or longer (Ptacek *et al.*, 2014).

2.3.1 Seasonality of reproduction

Sheep are seasonally poly-oestrus animals; meaning that they have a natural tendency to be more sexually active in certain times of the year than others (Mitchell *et al.*, 2002). Sheep breeds in temperate zones have adopted a short-day breeding strategy, i.e. they breed at times of the year when the photoperiod duration (i.e. number of daylight hours) is short, hence the term short-day breeders. Merino, Dorset Horn and Rambouillet are breeds that have developed in temperate climates; and can be considered typical examples of animals that express oestrus and anoestrus at different times of the year. In these breeds, oestrus is observed in autumn and winter, and a period of anoestrous (non-breeding season) in the spring and summer months (Marshall, 1937; Hafez, 1952). Reproductive performance and activity in ewes are influenced by two obvious rhythms. These rhythms include the ewe's oestrus cycle and the season-dependant anoestrus of the ewes. These rhythms in adult, non-pregnant ewes are both synchronized to produce offspring as well as to allow for reinitiating ovarian ovulatory cycles (Goodman, 1994; Gordon, 1996; Rosa and Bryant, 2003; Rawlings and Bartlewski, 2007).

The duration of the anoestrus period in ewes varies among breeds and individuals within a breed. More prolific breeds tend to have shorter anoestrus periods than non-prolific genotypes (Hafez, 1952; Webster and Haresign, 1983; Jeffcoate *et al.*, 1984; Goodman, 1994; Bartlewski *et al.*, 1998, 2000). This seasonal breeding nature of sheep thus ensures that lambs are born at the most ideal time of the year when there is an abundance of grazing opportunity for the ewes to support lactation. This dependence on the seasonal availability of pasture potentially presents a challenge to modern farming practices that are trying to best meet the consistent market demands and production costs.

2.3.2 Neuro-endocrine control of seasonality of reproduction

Nutrition and photoperiod effects the sexual maturity of the ewe through a common mechanism involving control of LH secretion (Butler, 2014). Puberty does not occur at a fixed critical weight, but is more likely to occur above a certain weight (Petrulis, 2013). Nutritional stress could delay follicular development and the preovulatory surge of luteinizing hormone (LH), which would affect the onset of puberty in ewes (Daley et al. 1999; Abecia et al. 2006; Petrovic et al. 2012).

Reproduction in sheep are greatly influenced by sensory inputs also known as sociosexual signals that influences physiological mechanisms as well as behaviour. The sociosexual stimuli when an ewe comes in full contact with rams activates LH and GnRH secretion. This neural activation response of ewes to the ram has been found to be not only accumulated by the scent or odor of a male. Sexually naive females have been found to only be affected by the ram when in full contact (Hawken and Martin, 2012). Nutrition may affect reproduction in sheep this way. Short-term nutritional deficiency might impair oestrus behaviour in sheep via the induction of an increase in foraging behaviour (J. García et al., 2016).

Melatonin is a hormone produced by the pineal gland that is responsible for the seasonal reproductive pattern observed for sheep breeds in temperate zones. Reiter (1974) stated that melatonin controls the seasonal breeding activity in short-day and long-day breeders through a direct or indirect influence on the hypothalamus. The retina is the photic sensor that transmits light signals along the retina-hypothalamic tract to the suprachiasmatic nuclei in the hypothalamus. During darkness, the sympathetic activity increases resulting in the increased secretion rate of melatonin. Signals generated by these nuclei are transmitted to the superior cervical ganglia and then to the pineal gland via sympathetic nerves. The pineal gland is the mediator between neural signals and the endocrine system that regulates cyclic reproductive activity.

Concentration of melatonin levels in the blood of the ewes as well as in the pineal gland are high at night, when exposure to photic energy is low, and conversely low during the day, when

exposure to photic information is higher. Long days are thus characterised by a short duration of melatonin secretion while short days are characterised by a longer duration of melatonin secretion. Secretion of melatonin thus follows a typical circadian rhythm (Yoshimura, 2013). The increase in melatonin secretion activates the hypothalamic-pituitary-gonadal axis, which in turn initiates the reproductive cycle. An increase in duration of daily melatonin secretion is associated with an increase in GnRH secretion, and subsequently an activation of the gonads in short-day breeders, whereas the same melatonin signal is followed by a regression of the gonads in long-day breeders (Grosse *et al.*, 1993).

Some experimental evidence also found that exposure of short-day breeding animals to light at night readily suppresses the secretion of melatonin (Lincoln, 1992). Towards the end of winter, anoestrus is triggered by prolonged periods of melatonin secretion. The ewes become desensitized by a higher concentration of melatonin, rather than a lower concentration of oestrogen and progesterone or even the lack of synchrony between follicular wave emergence and FSH peaks. The transition from oestrus to anoestrus are more likely due to the gonadal axis not responding to gonadotrophic hormones (Bartlewski *et al.*, 1999d). This lack of response is referred to as the animal becoming photorefractory, i.e. insensitive to photic stimuli. This is proved in in studies by Karsch *et al.* (1986) and Thimonier (1989), where Ile-de-France and Suffolk ewes were exposed to a prolonged period of short days. Breeding activity for these ewes usually begin 50 days after the transfer from longer to shorter days, and the season lasts for about 60-80 days. The ewes became refractory to shorter days after about 120 days.

During this photorefractory period, follicular wave emergence could not be initiated by peak serum follicle stimulating hormone (FSH) concentrations. Significant lower levels of oestrogen and progesterone (produced by the *corpus luteum*) were found, when compared to the levels of these hormones during mid-breeding season (Bartlewski *et al.*, 1999a, 1999d). During summer, which is characterized by long daylight hours, melatonin secretion decreased and serum levels were too low to trigger oestrus. However, these lower concentrations of melatonin prepare (condition) the ewes to become susceptible to higher concentrations of melatonin again. When the duration of photoperiod shortens again, natural cycling will start, where higher melatonin concentrations will initiate the onset of oestrus again.

2.4 The breeding cycle of the ewe

2.4.1 Oestrus

The oestrus cycle of the ewe is characterized by four phases, i.e. proestrus, oestrus, metestrus, and dioestrus. The length of the ewe's oestrus cycle is on average 17 days and

range from 13 to 19 days in duration. The first day of the oestrus cycle is assumed to be when ovulation occurs, with the -cycle ending just prior to the next ovulation. Anoestrus is referred to as the condition where there is no cycle expressed, i.e. a state where no follicular and ovulatory activities are observed in or on the ovaries.

During proestrus, the corpus luteum (CL) will decrease in size and the amount of progesterone in blood will also decrease. Proestrus will usually extend from day 4 to day 13-15 of the oestrus cycle. During oestrus, the follicles will grow rapidly until ovulation. Metestrus is the period where ovulation ceases, and the formation of the CL. Metestrus usually lasts for approximately 3 days. Diestrus is the period where the CL is fully functional. The CL secretes progesterone, which will be used for the maintenance of pregnancy if fertilization and implantation were successful.

A complex system of regulatory hormones controls the oestrus cycle (Figure 2.3). These hormones include gonadotropin-releasing hormone (GnRH), FSH, luteinizing hormone (LH), oxytocin, oestrogen, inhibin, progesterone, prostaglandin F2 alpha ($PGF_{2\alpha}$). The inter-related relationship between these hormones and how they influence the oestrus cycle, is regulated by a collaboration between the hypothalamus, the pituitary gland, ovarian antral follicles, corpus luteum and the endometrium of the uterus (Scaramuzzi *et al.*, 1993a, 1993b).

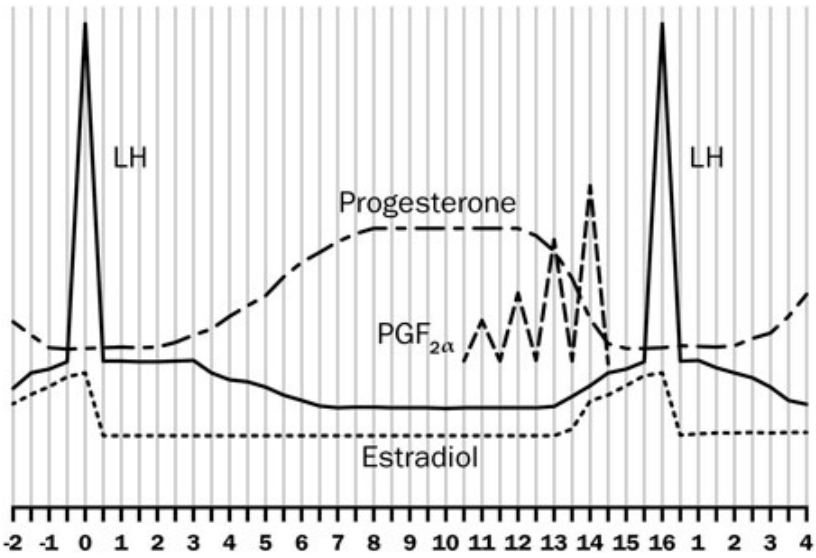

Figure 2.3. Hormonal control of the oestrous cycle in sheep (Sheep Production Handbook, 2002).

Briefly, the hypothalamus produces GnRH, which is transported to the anterior pituitary gland, which in turn secretes FSH and LH, that are transported to the ovaries. The FSH and LH will then act on the ovaries to initiate and support follicular development, and ultimately ovulation.

A prerequisite for antral follicular growth and maturation is that the developing follicles are responsive to an increasing level of gonadotrophic hormones (Campbell *et al.*, 1995).
The serum levels of GnRH, FSH, and LH will reach a peak concentration 14 hours before ovulation, with serum LH concentration being higher than that of FSH prior to ovulation (Baird, 1978; Rawlings *et al.*, 1984). This peak in LH is a direct result of the increase in oestrogen production by the Graafian follicle, which is also considered as a pre-ovulatory in size, that when observed with ultrasound, will be a minimum of 6 mm in diameter (Teixeira et al., 2008). The LH peak will ultimately result in the release of an ovum from the Graafian follicle, that ruptures due to a combination of an increase in intra-follicular pressure caused by an expanding of the cumulus cells-ovum complex, and a weakening of the membrane in the region of the stigma by $PGF_{2\alpha}$ (Iain J. Clarke, 2014).

After ovulation occurred, the Graafian follicle space will be filled with blood, resulting in the formation of the *corpus haemorrhagicum*, which in turn will be converted in the CL, whose initial growth is dependent on LH (Niswender *et al.*, 2000). The CL can clearly be observed growing to a size of between 6-8mm in diameter, about 3- 4 days after ovulation, reaching a maximum size of between 11- 14mm in diameter, 9- 10 days after ovulation (Bartlewski *et al.*, 1999b). The CL produces progesterone, which is conditional for the maintenance of pregnancy. If fertilization and implantation were unsuccessful, $PGF_{2\alpha}$ will cause the CL to degenerate between days 12 and 15 after ovulation (Bartlewski et al., 2011a). The higher levels of progesterone communicate to the hypothalamus to thus decrease GnRH secretion. A prerequisite for antral follicular growth and maturation is that the developing follicles are responsive to an increasing level of gonadotrophic hormones (Campbell *et al.*, 1995).

Progesterone and oestrogen works together to regulate the frequency and intensity of LH pulses, with the secretion of LH being inversely related to the circulating progesterone level (Bartlewski *et al.*, 2000; Duggavathi *et al.*, 2005b; Barrett *et al.*, 2007). The low LH levels and high progesterone concentration in the blood, will thus suppress follicular growth, which will remain suppressed until progesterone levels decrease below the serum concentration that will eventually result in the initiation of follicular development in the ovaries again.

2.4.2 Ovulation

Ovulation usually is timed to occur in the window opportunity that will allow for the spermatozoa and the ovum to meet at the most optimal time for fertilization in the ampulla, i.e. the time between display of signs of standing heat that is a direct result of the peak oestrogen production by the Graafian follicle that is of ovulatory size, which in turn will signal the ram that

the ewe is ready to be mated. This “timing of mating” allows the sperm to be present in the oviduct by the time the ovum reaches the site of fertilization. Ovulation occurs usually 24-27 hours after the first signs of standing heat, which allows the ovum a 10-25 hour window of opportunity to be fertilized by the sperm, which can survive for about 30 hours after mating.

Ovulation rate is the primary determinant of fertility and fecundity in female animals (McDonald *et al.*, 1995). The establishment of ovarian cyclic activities during puberty is condition for the sexual maturation and reproductive success of a ewe. Unlike camels or rabbits where ovulation is triggered by copulation, ewes ovulate spontaneously and also have the ability to manipulate the number of ova released during each cycle of ovulation. This ability to manipulate their own reproductive output serves as a function of their perception of the immediate nutritional environment, and the potential survival of their offspring (O’Connell *et al*., 2016).

2.4.2.1 Factors influencing ovulation rate

Ovulation rate can be influenced either in the period of recovery between lactation and mating, or during the period of mating. The loss of ova can be affected by the plane of nutrition during the recovery period between lactation and the next mating, and prenatal mortality can be affected by the resources a ewe has available to invest in lactation (Coop, 1966). There are thus two aspects of nutrition that play a role in fertility, i.e. the static and dynamic effects that are linked to specific time periods. The static effect of nutrition occurs during the recovery period (i.e. between lactation and the next mating). The dynamic effect is evident in the conditioning period of the three to four weeks preceding mating, which is also known as the flushing period. Follicular recruitment, growth, and maturation that will eventually result in successful ovulation requires a period of six months from the point that follicles are recruited from the pool of primordial follicles (Cahill and Mauleon, 1980; Driancourt and Cahill, 1984). Both the static and dynamic effects of nutrition influence lambing rate (Coop, 1966).

Nutritional handicaps experienced at any stage during follicular development up and to ovulation, influence ovulation rate in the ewe. Fletcher (1974) reported a reduced ovulation rate in response to restricted feed intake in the six months prior to ovulation. According to Nottle *et al.* (1997), restricting nutrition six months prior to ovulation may affect the ovulation rate on three levels, i.e. fewer follicles will be recruited from the primordial pool and commence growing, subsequent follicular development will be inhibited, or some follicles that would have normally reach ovulatory size will fail to do so, and thus be lost through atresia.

During the prenatal development of the female foetus, the primordial pool of follicles that have the ability to be recruited for development, is established (Bearden and Fuquay, 1997). Oogonia are produced through mitotic division of the primordial germ cells in the foetus's ovaries. Mitosis in the ovaries ceases at birth, with the total pool of follicles that can be recruited from, already established at birth. Meiosis will occur soon after birth of the female lamb, which is initiated by factors released from the *rete ovarii*; and will cease for a period of resting. After the onset of puberty, meiosis again resumes in the ovaries (Knobil and Neill, 1988). The structure of a primary follicle appears to be a germ cell surrounded by a single layer of follicular cells. Most follicles undergo atresia after the first stages of development, with some that actually reach full maturity, to yield an ovum that is released during ovulation to be fertilized in the ampulla (Bearden and Fuquay, 1997).

During an oestrus cycle of an ewe, there appears to be a wave-like pattern of ovarian follicular development, which is a progressive and recurring process, and with two to three waves occurring during each cycle. The wave of follicular development is characterized by a group of small follicles that is recruited to undergo growth, with this phase of pre-antral follicular development taking place from approximately six months to six weeks before ovulation. The final stage of antral follicular development is also characterized by either ovulation or atresia. In some animals, there exist a kind of hierarchy in the follicular wave, with one follicle (i.e. usually in the last wave of a cycle) that are dominant. The dominant follicle is the one targeted for ovulation, with the subordinate follicles that will undergo degeneration through atresia. It is however not uncommon for two or three follicles to mature to an ovulatory size in sheep, with the follicular dominance mechanism not as evident in sheep as in cattle (Driancourt *et al.*, 1991b).

Nutritional restrictions during the antral phase of follicular development have been found to have a negative effect on ovulation rate (Coop, 1966; Killeen, 1967; Fletcher, 1971). Driancourt and Cahill (1984) agreed with the findings of Coop (1966), Killeen (1967) and Fletcher (1971), when they stated that an increased incidence of follicular atresia may be the result of underfeeding prior to ovulation, resulting in less follicles available for ovulation. Apart from the ovulation rate, it is reported that lambing rate is affected by the fertilization rate and embryo survival (NRC, 1985). Embryo survival in turn is influenced by the body condition of the ewe, which in turn is determined by the plane of nutrition of the ewe.

2.5 Manipulating of the reproductive cycle of the ewe for improved economic returns

When extensive sheep production systems are compared to more intensive approaches, productivity and profitability will be determined by the viability, sustainability, and optimal management of these systems. In South Africa, it is common practice for extensive systems to produce a minimum of one offspring/ewe/lambing season that can be marketed, which is also dependant on the seasonality and patterns of pasture or veld growth (Nel, 1980; de Nicolo, 2007). In intensive sheep production systems, the focus is on an increase in the stocking rate by increasing the quality and quantity of feed provided to the animals, e.g. using cultivated pastures or full ration feeds to increase the total output per unit (Bouwman, 2007). To potentially improve the economic return through an improvement in lambing percentage and thus decrease input costs, the timing of breeding in ewes can be manipulated through the use of three natural processes or factors, i.e. photoperiod, nutrition, and the ram effect.

2.5.1 Use of artificial lighting programs

Artificial light manipulation is one of the first thought-of solutions to manipulate the breeding season. A ewe's seasonal rhythm can be manipulated naturally or artificially by a period of artificial long-days just before the daylength (i.e. of photoperiod) become shorter again. This manipulation bypasses the neuro-endocrine mechanisms in a sheep's brain, when sheep is supposed to become photorefractory. This manipulation will successfully reinitiate the oestrus cycle to start again. This artificial exposure has a more pronounced effect when combined with the "ram effect". To ensure the successful use of the management intervention, a farmer will thus require a facility where the light exposure of the ewes can be controlled (Ortavant *et al.*, 1988).

2.5.2 Use of a teaser ram

Another natural manipulation of the oestrus cycle is the "ram effect". The ram effect is a well-established method of stimulating ovulation and oestrus in anovulatory ewes (Martin *et al.*, 1986; Rosa and Bryant, 2002; Ungerfeld *et al.*, 2004). The ram effect entails the introduction of a sexually mature and active ram to anoestrus ewes, either in the period prior to anoestrus or in the period just before the natural oestrus cycle is about to end. This will result in a rapid increase in FSH and LH, which will be in reaction to a surge in GnRH secretion by the hypothalamus in the ewes. The potential of this method to synchronize oestrus activity in ewes can be limited by the seasonal nature of reproduction in breeds in temperate regions (Lindsay and Signoret, 1980). The best response to the ram effect will thus be achieved and observed

close to the onset of the natural breeding season in such seasonal breeds (Rosa and Bryant, 2002).

The response of the ewes to the introduction of the ram is evident in the initiation of and improved follicular development and maturation, and potentially also out of season ovulation. The ram effect is known for extending the natural breeding season by approximately one month either before or after the natural time. For this management practice to be successful, ewes need to be maintained visually and physically separate from the rams for a period before the ram is introduced. Ewes maintained in continuous ram contact can become habituated or resistant to the ram effect (Martin *et al.*, 1986). It is commonly known that the ram effect will be accompanied by a first ovulation that are silent. Thus, for the ram effect to be successful, the ram have to remain with the ewes until all of them have had their first ovulation, and successful being mated, which can only be observed after about 18 to 24 days.

The stimulatory influence of the ram effect is realized through a collaborative effect of the olfactory stimulation by the ram's pheromones that are found to occur on the wool and around the ram's eyes and flanks, and non-olfactory stimuli such as ram behaviour (e.g. showing interest in a ewe). The experience of the animals of both sexes have also been found to have an effect, with younger rams that may not elicit a pronounced response from sexually mature ewes (Rosa and Bryant, 2002).

2.5.3 Use of flush feeding

Nutrition is widely acknowledged to interact with the seasonality and cyclicity of reproduction in sheep, likely through the central hypothalamic pathway. Nutritional status of the ewe, before or after ovulation, may affect oocyte development, embryo development, and successful establishment of pregnancy (Sen *et al.*, 2013; de Brun *et al.*, 2016). The number of ova fertilized as well as embryo survival are both factors that are influenced by the nutritional status of the ewe prior to and during mating, and in turn the latter two factors influence the lambing rate (NRC, 1985).

Underwood and Shier (1941) stated that flushing encourages the maturation of a larger number of ova. Flush feeding animals is a practice that aims to increase the overall reproductive performance of the flock by positively influencing the reproductive behaviour and body condition of the ram and ewe, and ultimately conception rate and/or lambing rate. Ocak *et al.* (2006) found that short-term supplementation (i.e. 15-17 days) post-mating can beneficially influence the non-return rate as well as lambing rate after the first oestrus of a ewe, as well as litter size. The general definition of flushing is to increase the plane of nutrition

prior to and during breeding, by ensiring that the ram and ewe has access to an excess of protein and energy in the diet, which can then be partitioned to the physiology taxing physiological processes associated with reproduction (Miller, 1913; Bray, 1925; Hultz and Hill, 1931; Cooper, 1933; Spencer, 1939; Underwood and Shier, 1941; Richards, 1942; Lush, 1945; Anderson, 1947; Reeve, 1953; Watkins, 1955; Ballinger, 1956; Pope *et al.*, 1956).

Rattray (1982) postulated that an increase in body weight prior to mating will result in an increase in the number of ovulations during the mating period, which will improve the reproductive potential of the animal and ultimately the flock. Breed and age of the ewe are both factors that affect the response to flushing, with mature ewes responding better than younger ewes. There are however, factors such as the type of protein and energy sources included in the flushing diet, intensity of flushing, the duration of the flushing period, body condition of the ewe, and the effect of season, that all can determine how pronounced the reaction to flush feeding may be (Coop, 1966; Haresign, 1983; Loubser, 1983; Rhind, 1987).

Coop (1966) used the terms “static” and “dynamic” to describe the nutritional effect on reproduction. The static effect is described by assessing the body condition, live weight and/or size of the ewe. The dynamic effect is defined as a change in live weight, which in turn will be reflected in a change in body condition, e.g. what can be experienced with flush feeding prior to and during the mating period. The combination of the static and the dynamic effect represent a more accurate indication of a ewe’s nutrient reserves. Live weight alone on the other hand is too insensitive a parameter, seeing that it just is a combination of body size and body condition. Ducker and Boyd (1977) reported for ewes with the same body condition, body size had no effect on mean ovulation rate. In Scottish Blackface ewes, Gunn and Doney (1975) found a positive linear relationship between ovulation rate and body condition at mating.

Lindsay (1976) defined the “net nutritional status”, which is best described as the sum of the nutrients absorbed daily from the digestive tract and the nutrients available from body reserves. He thus suggested that ovulation rate in ewes is more related to her net nutritional status. Under- and over-feeding post-mating may result in a lower yield of lambs born (Doney and Gunn, 1981). Some nutritional components are known to affect the ovulation rate of ewes, without affecting ewe live weight (Knight *et al.*, 1975; Smith *et al.*, 1979). A good example is lupins supplementation to the ewes prior to ovulation or mating. Response to lupins supplementation includes an increased ovulation rate, without a measurable increase in live weight (Lightfood and Marshall, 1974; Knight *et al.*, 1975). Pearse *et al.* (1994) confirmed these finding when he reported a 64% higher ovulation rate in a lupins-supplemented group of ewes who maintained their body condition during their study. These results indicate that

regardless of body condition, nutritional flushing will have a stimulating effect on the ovulation rate in ewes.

2.6 Partitioning of diet components during the flushing period

A standard flushing diet is generally formulated to provide more energy and protein to the ewe or ram than what she/her will require for daily maintenance activities. The animal thus have the opportunity to repartition these two feed components towards physiological taxing process of reproduction.

2.6.1 Partitioning of energy in the ewe

According to Wentzel (1986), ovulation rate responds favourably to short-term high energy intake (such as with the feeding of a flushing diet) only when the ewe has an intermediate body condition score of two to three. Wentzel (1986) also found that 48 hours after the start of the feeding of a flushing diet, a 60% increase in blood glucose concentration occurred. Russel (1978) and Erasmus (1990) postulated that blood glucose levels is a fair indicator of the energy status of the animal. According to Venter and Greyling (1994), the higher energy available as a result from flushing results in a higher blood glucose concentration, and this impacts positively on the reproductive performance of the ewe by stimulating the anterior pituitary gland to release more LH than normal, which improves the ovulation rate.

Successful flushing relies on two prerequisites, i.e. firstly the ewes must experience a significant weight gain in response to flushing, and secondly the nutritional plane of the ewe needs to be lower than the natural nutritional plane that would have resulted in the highest return in lamb crop for the breed in question (Marshall and Potts, 1924; Darlow and Hawkins, 1933; Clark, 1934; Friedman and Turner, 1939). The ewe to be stimulated needs to have a more pronounced drive for feed intake than mating. Haresin (1983) found that at mating, that the feed intake of ewes with a BCS of one to two, was 35% higher than ewes with a BCS of three to four.

Pearse *et al.* (1994) reported results that countered the influence of ewe body condition before flushing, and suggested that a flushing diet may increase ovulation rate regardless. The source of the flush feed and the level of protein and/or energy and the timing may have critical consequences on the reproductive efficiency in sheep (Parr *et al.*, 1987; Rhind *et al.*, 1989; Molle *et al.*, 1995; Landau *et al.*, 1996; Abecia *et al.*, 1997; Molle *et al.*, 1997; Branca *et al.*, 2000). For optimum follicular development and embryo development respectively, the nutrient requirements of the ewe may also differ (O"Callaghan and Boland, 1999). Low feed intake prior to the mating season reduces the mean ovulation rate, and low feed intake post-mating

resulted in a slower embryo growth rate and increased the number of ova lost through atresia (Rhind *et al.*, 1989).

Marshall and Potts (1921) reported that there is generally no difference in the kind of feed used for flushing, e.g. pasture or grain, as long as the two abovementioned prerequisites are met in a flock. Any fodder that is of good quality and palatable is recommended for flushing (Bray, 1925; Geary, 1956). An improved potency of FSH and LH was the result of grain feeding that increased ewe weight, with a concomitant increase in plasma glucose levels and heavier adrenal and pituitary weights recorded (Bellows *et al.*, 1963; Howland *et al.*, 1966; Memon *et al.*, 1969). O'Callaghan *et al.* (2000) stated that in super-ovulated ewes, severe dietary energy restrictions can alter follicle growth characteristics. Bean and Butler (1997) studied the development of post-partum dominant follicles and found that despite a negative energy balance, that the follicles were resilient to periods of energy deficiency.

The level of energy in the diet of a sheep plays a role in the metabolism of protein in the diet. Available fermentable and metabolized energy is a prerequisite for the conversion of rumen degradable protein into microbial protein. If the energy level is insufficient to convert rumen degradable protein to microbial protein, surplus ammonium ions are converted into urea by the liver and removed from the blood via the kidneys. If there is sufficient energy present, microbial protein is formed that is digested further along the digestive tract, which in turn will be available to the animal after uptake in the small intestine for utilization in several biochemical process, amongst others, the production and secretion of hormones that play a crucial role in the initiation, support, and success of reproduction (Iain J Clarke, 2014).

2.6.2 Partitioning of protein in the ewe

Richards (1942) indicated that the addition of phosphorus in a flushing supplement to sheep maintained extensively, will increase the lambing rate indefinitely. Harris *et al.* (1956) on the other hand, found that supplementation for range feeding works better in increasing the lambing rates when phosphorus and protein are combined. Van Horn *et al.* (1952) stated that higher protein levels in the diet would have the biggest influence in this regard. Miilin (1924) indicated that it is a well-known fact among sheep producers that ewes gaining weight during a mating season, produce more lambs when compared to ewes losing weight or that maintained their condition.

Work by Fletcher (1981) and Davis *et al.* (1981) studied the effects of protein on the ovulation rate of ewes. Fletcher (1981) reported that with low levels of dietary energy (4 MJ ME/ewe per day), a response was observed when dietary protein was increased. Davis *et al.* (1981)

reported concurring results, where at moderate energy levels (11.1 MJ ME/ewe per day), ewes responded favourably to a higher protein inclusion level. However, in their study, when ewes were fed a diet with a low energy level of 6.25 MJ ME/ewe per day, ewes failed to respond even when the diet was characterized by a higher protein inclusion level. An increased ovulation rate resulted after a high protein supplement were fed to ewes for 32 days compared to the ewes not receiving more protein, with the live weights remaining constant for both groups (Davis *et al.*, 1981). Fletcher (1981) and Davis *et al.* (1981) reported a response to an increased dietary protein at a constant level of dietary energy intake.

Van der Westhuysen (1971) studied the interaction of age and level of dietary protein in ewes. He stated that embryonic mortalities increased in maiden ewes that were fed low levels of protein, compared to mature ewes. Bennett *et al.* (1964) stated that while mature ewes are more resilient to malnutrition, the lambing percentage of 2-year-old primiparous ewes significantly declined in response to underfeeding, which confirmed the findings of Van der Westhuysen (1971).

Ocak *et al.* (2006) found a positive response in non-return rate, lambing rate and litter size in primiparous ewes grazed on rangeland, in response short-term (15-17 days) supplementation of protein. Previous studies reported that supplementation with lupins (Downing *et al.*, 1995; Nottle *et al.*, 1997a), soybean meal (Molle *et al.*, 1995, 1997; Branca *et al.*, 2000) or groundnut seed cake (El-Hag *et al.*, 1998), particularly during the pre-mating period, induced an increase in reproductive performance.

The effect of supplementary feeding on reproductive performance (Rhind *et al.*, 1989; O'Callaghan and Boland, 1999; Branca *et al.*, 2000) may be the direct or indirect effect of poor rangeland (Molle *et al.*, 1997; Santra *et al.*, 2002; Chaturvedi *et al.*, 2003). A study by Ocak *et al.* (2006) showed that a short-term (2 weeks) increase in level of protein supplementation during the post-mating period improved reproductive performance in ewes maintained on rangeland. The findings discussed above thus suggest that formulating diets with a high protein level potentially presents an approach to improve the reproductive performance of ewes when rangeland quality decreases, even after mating.

2.6.3 Partitioning of fatty acids in the ewe

The inclusion of polyunsaturated fatty acids (PUFAs) in the flushing diet improves the fertility of sheep (Journal and Kili, 2020). The fatty acids that have an influence on ruminant reproduction, include the long-chain n-3 and n-6 omega fatty acids that are synthesized by the ruminant through the desaturation and elongation of short-chain n-3 linolenic acid (ALA, 18:3n-

3) and n-6 linoleic acid (LA, C18:2n-6). These two fatty acids are termed essential fatty acids, and need to be included in animal diets (Nieto *et al.*, 2015). Sources of ALA and LA that can be included in ruminant diets are found in certain forages and linseed. Long-chain n-3 fatty acids (20 carbons or longer) found in fishmeal and fish oil, are also suitable to be fed to ruminants (Ashes *et al.*, 1992). Short-chain LA can be found in various raw materials including grains, soybean, safflower and sunflower. Commercially products are available that contains as much as 10% of total lipids as n-6 LA, e.g. Megalac (Gulliver et al., 2012b).

The omega-3 and n-6 PUFAs can influence the reproduction of sheep through a beneficial effect on metabolism and/or synthesis of progesterone (P4) and oestradiol (E2). Some studies found the n-3 fatty acids had a negative impact on steroid synthesis, due to the fact that diets high in n-3 ALA are associated with lower plasma cholesterol concentrations, with the latter that is required as precursor for the synthesis of P4 and E2 (Staples *et al.*, 1998; Robinson *et al.*, 2002). Other studies found that the inclusion of n-3 acids in feeds has an inhibitory effect on the working of $PGF_{2\alpha}$ in ruminants, which in turn prevents the CL to undergo regression, which in turn will result in sustained P4 release and thus an extended inter-calving interval (McCracken *et al.*, 1972).

The potential beneficial influence of flushing diets formulated to include ALA and LA, can be determined by evaluating the influence of such diets on ovulation rate and oocyte viability, which in turn will be determined by the number and size of ovulatory follicles on the ovaries (Ambrose *et al.*, 2006). Contradictory results, however, were reported for the inclusion of n-3 and n-6 fatty acids in the diets of dairy cattle. The mean diameter of ovulatory follicles and CL were found to be larger than the control group for the cows fed n-3 fatty acids and lower than the control group for cows fed n-6 fatty acids (Homa and Brown, 1992; Petit *et al*., 2002; Ambrose *et al*., 2006; Mendoza *et al*., 2011). However the effect of the inclusion of n-6 fatty acids in beef cattle rations was positive with an increase in the number of medium sized follicles (Thomas *et al*., 1997)

Feeding costs comprises approximately 70% of input costs, and should expensive raw materials such as n-3 and n-6 fatty acids be included in breeding diets, the question arises whether inclusion of these fatty acids will be reflected in an improved reproductive performance, and ultimately an improved lambing percentage and thus increased returns. One of the main motivations for this study was that commercial feed companies formulate so-called “super-fertility” diets that are alleged to improve the overall reproductive performance of breeding animals. Inclusion of ALA and LA is potentially one approach that can be followed

for the formulation of such “super-fertility” diets, however due to the confidential nature of the formulation specifications, companies will not disclose their diet specifications.

2.7 Ultrasound scanning as a management tool to monitor and evaluate follicular development

B-mode ultrasonography is considered a valuable management tool to detect pregnancy in livestock species (Garcia *et al.,* 1993; Toosi *et al.,* 2009; Barbagianni *et al.,* 2016). Ovarian ultrasonography is also a useful tool to track the progression of antral follicular kinetics, ovulation, and the formation of the CL (Schrick *et al.*, 1993; Gonzalez-Bulnes *et al.*, 2000; Duggavathi *et al.*, 2003a, b; Vi˜noles *et al.*, 2004). The growth profile for large follicles (i.e. larger than 1-2mm) and the CL can be studied using transrectal ultrasound scanning of ewes. For smaller follicles the predictive value and sensitivity of ultrasonography are lower (Viñoles Gil, 2003).

The accuracy of ultrasonography may also decrease with a larger number of CL on the ovaries (Dickie *et al.*, 1999). Carolina Viñoles (Gil, 2003) also found that to be true in the cases where a high number of CL was present, the sensitivity of ultrasonography decreased, when compared to cases where one CL was observed. The low incidence of more than one CL and human error thus needs to be considered when calculating the sensitivity of ultrasonography where one error can largely effect the results. It is a common mistake where a large central cavity in the uterus are mistaken for a large follicle during the scanning protocol after oestrus have commenced in the ewe. In cases like the latter, the number of CL may be underestimated. Similarly, the number of CL may be overestimated in the cases where non-functional CL are still present from the previous oestrus cycle.

By scanning ewes at a later stage, e.g. 10 days after oestrus, the limitations of ultrasonography can be overcome when a well-developed CL can be clearly identified (Dickie *et al.*, 1999). Regarding follicle size, the sensitivity of ultrasonography are lower for follicles between 2-3mm in diameter (Viñoles Gil, 2003). Viñoles Gil (2003) found that when follicles ranging between 2-6mm in diameter were recorded that the majority of the follicles were 2 mm in size. Considering that the recruitment of follicles at wave emergence are identified by the difference in number of follicles between 2mm and 3mm in diameter, the abovementioned is crucial to take into account in the recording and interpretation of ultrasound results. Discrepancy of recording of this size follicles occur, since it is difficult to accurately determine these follicles (<4mm) by ultrasonography and may also be ascribed to inconsistency between investigators. Despite this, ultrasonography is still considered an valuable tool for studying follicular

dynamics, since follicles ≥4 mm are identified with high accuracy using this technique (Viñoles Gil, 2003).

The classification and features of ovine ovarian follicles from the reserve pool of primordial follicles until the formation of antral follicles, with estimated durations of various stages and growth rates (changes in diameter) during the progression of folliculogenesis, is presented in Figure 2.4. Each scale bar on the histology images depicting microscopic follicles (Warren *et al*., unpublished) represents 25µm and a single scale bar inserted in the ovarianultrasonogram (lower panel; Bartlewski *et al*., 1998) represents 1mm.

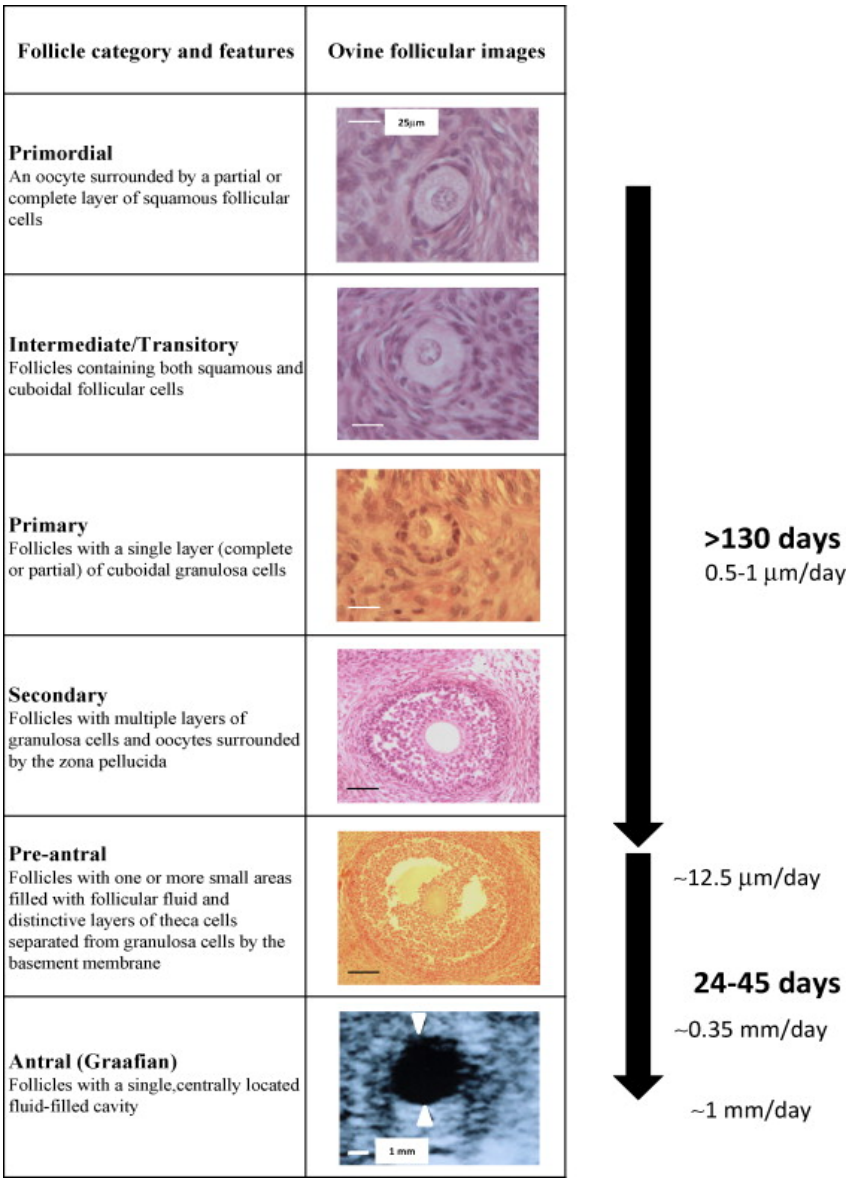

Figure 2.4. Classification and features of ovine ovarian follicles (Huchkowsky *et al*., 2002)

Transrectal ultrasonic imaging led to the technological breakthrough and made the study of follicle development in ruminants possible (Pierson and Ginther, 1984). Visualization of

ovarian follicles ≥2 mm in diameter could be determined and the growth rate of follicles from recruitment until it reaches pre-ovulatory size was also made possible (Bartlewski *et al.*, 1999; Evans *et al.*, 2000).

The number of follicles that progress from one growth phase to the next, decreases with each phase, with most follicles lost through atresia. This loss in follicles through atresia contributes to the regulation of the number of ova that are released during an oestrus cycle. The phases of follicular development are collectively referred to as “waves of follicular development”, which are regulated by gonadotrophins to take place in an organized and cyclic manner. The different growth phases during an ovulatory wave are described below.

2.7.1 The five functional classes of follicles and corpus luteum

2.7.1.1 Primordial follicles

Primordial follicles make up the majority of non-growing follicles, which gradually deplete during the ewe’s reproductive lifespan (Greenwald and Shyamal, 1994). Figure 2.4 shows a high density of small primordial follicles present in the ovaries. Primordial follicles are known as oocytes where the zona pellucida (ZP) has not formed yet, and which are surrounded by a flattened layer of pre-granulosa cells. These follicles also lack blood capillary networks (Scaramuzzi *et al.*, 1993).

2.7.1.2 Committed follicles (Secondary follicles)

Once the primordial follicles are recruited, irreversible growth occurs in an organized sequence. Follicles grow very slowly before antrum formation (i.e. very slow phase of growth), taking 130 days to reach the next phase of growth (Cahill and Mauleon, 1980). In this phase, the main change observed is the development of the zona pellucida around the oocyte. Theca cells are also observed once two to three layers of granulosa cells are formed (Scaramuzzi *et al.*, 1993). Steroid-secreting theca cells are formed from the differentiation of stroma cells through the action of Insulin-like growth factor II (IGFII) (Perks *et al.*, 1995). FSH receptors are identified on granulosa cells, and LH receptors on the theca cells of follicles of this size class. Autocrine and paracrine factors influence the growth of these primary follicles to a larger extent than gonadotrophins (McNeilly *et al.*, 1991; Findlay *et al.*, 2000).

2.7.1.3 Gonadotrophin-responsive follicles (Pre-antral follicles)

Follicles grow at a slow rate (slow phase of growth) after antrum formation, taking 30 days to grow from 0.2 mm to 0.7 mm in diameter. Faster growth follows at the end of this stage, where follicles increase in size from 2.5mm to 8mm in 5 days (i.e. fast phase of growth). Rate of

granulosa cell development reaches a maximum when the follicle is 0.85 mm in diameter followed by a decreased rate (Turnbull *et al.*, 1977; Cahill and Mauleon, 1980). Aromatase activity starts at this stage and plays an important role in follicle development, through its role in steroidogenesis.

Significant oestradiol concentrations are only found after the follicle reaches 0.5 mm in diameter (Scaramuzzi *et al.*, 1993). Sensitivity of granulosa cells to FSH increases with an increase in aromatase activity (Scaramuzzi *et al.*, 1993). Insulin-like growth factor I (IGF-I) stimulates the production of granulosa cells, and in combination with FSH it plays a role in granulosa cell differentiation (Monget and Monniaux, 1995; Poretsky *et al.*, 1999). Androgen can be found in the follicular fluid of most gonadotrophin-responsive follicles. and oestradiol in the more developed follicles that exhibits aromatase activity.

The size at which follicles are recruited to ovulate and the size of follicles that become gonadotrophin-dependent are linked and follows a specific sequence (Driancourt, 2001). The recruitment window for follicles in sheep lasts 36–48 hours (Souza *et al.*, 1998; Bartlewski *et al.*, 1999).

2.7.1.4 Gonadotrophin-dependent follicles (Antral follicles)

An absolute concentration of FSH are required for follicles to progress from gonadotrophin responsiveness to gonadotrophin dependency. Increased aromatase activity and oestradiol concentration is a normal consequence of a high enough serum FSH concentration. Luteinizing hormone receptors (LHrs) are found on the granulosa cells of this gonadotrophin dependant follicle. The joint action of oestradiol and FSH induced the LHrs (Richards, 1980). Androgen will accumulate within the follicle when FSH concentrations are inadequate to maintain aromatase activity, resulting in lower oestradiol secretion and subsequent atresia (Scaramuzzi and Campbell, 1990). Gonadotrophin-dependent follicles have a higher need for FSH than gonadotrophin-responsive and ovulatory follicles.

2.7.1.5 Ovulatory follicles

Even faster growth follows the growth stage of the gonadotrophin-dependant follicles (i.e. very fast phase of growth) culminating in an ovulatory follicle. Dowing and Scaramuzzi (1991) stated that ovulatory follicles are the most vulnerable to atresia.

For the development of a gonadotrophin-dependent follicle to an ovulating follicle to occur, a critical concentration of FSH is required (Campbell *et al.*, 1999). Ovulatory follicles have many

LHrs and FSH receptors (FSHrs) in their granulosa cells. The growth in diameter of an ovulatory follicle can be ascribed to the granulosa cell increase, which also leads to an accumulation of follicular fluid in the antrum of the follicle (Turnbull *et al.*, 1977). At this stage aromatase activity is maximal and intra-follicular oestradiol levels peaks for the ovulatory follicle (Hsueh *et al.*, 1984).

The ovulatory follicle is responsible for more than 90% of the circulating concentration of oestradiol (Baird and Scaramuzzi, 1976; Baird e*t al.*, 1991). Oestradiol synthesis is a prerequisite for the consistent development of the ovulatory follicle (Baird, 1983).

2.7.1.6 Corpus luteum

The CL is the only ovarian source of progesterone and other progestins. The CL is well supplied with blood vessels, and has a greyish-white colour. The CL does not regress until late pregnancy (Bearden and Fuquay, 1997). When regression occurs, progestin secretion decreases and eventually cease, after which the CL loses it colour and eventually appears as a small white scar on the surface of the ovary, after which it is referred to as a *corpus albicans*.

2.8 Factors influencing the reproductive efficiency of ewes

The reproduction rate for sheep is defined as the number of live lambs born, per ewe mated, and is likely the most important profit driver in a sheep production system. There are various factors that can influence the reproductive rate of ewes, which will be discussed below.

2.8.1 Out of season breeding

Intensified sheep production management led to the possibility for sheep to reproduce outside of the natural season when oestrus occurs, when they are supposed to experience anoestrus (Jeffcoate *et al.*, 1984; Rawlings *et al.*, 1987; Gordon, 1996). During mid-anoestrus it is common for some breeds to experience silent ovulations (Ortavant *et al.*, 1988). The transition from the anoestrus to oestrus is gradual, and during the transition period, the occurrence of short oestrus cycles is common, with the first CL that decreases prematurely 5 to 6 days after its development.

2.8.2 Stress

The main effect of stress on sheep reproduction is inconsistent, i.e. stress can increase or shorten the duration of an oestrous cycle, and even reduce the rate of ovulation (I. J. Clarke and Arbabi, 2016). Rosa and Bryant (2002) found that the rate at which ewes are mated can also be influenced by stress. Stress during gestation has also been reported to affect the

embryo viability and survival. Animal handling during gestational periods thus need to be minimized (Grandin and Collins, 2007). Nutritional stress during the initial stages of gestation usually has a positive effect on embryo survival, as long as the ewe does not loose condition (Nandi *et al.*, 2017).

2.8.3 Selection

Selection for multiple-bearing ability have shown to gain 3% of lambs born per year, per ewe mated (Turner, 1966). Uncertainty of exactly how much credit the genetics should get arises when, in the same study, there was a pattern of an increase in multiple lambs born with increase in age of the ewes in lambs born/ewe mated (Michels *et al*., 2000).

Pacham concluded that even though the ewe are the only party capable of expressing the gene, twinning ability are inherited by both the ram and the ewe (Pacham and Triffitt, 1966). Selection for good maternal qualities are often preferred to replacement animals born as twins or triplets. Multiples can slowly increase the prolificacy within a flock, however offspring that have desirable traits of better mothering ability and milk production might increase the prolificacy faster.

2.8.4 Live weight and body condition

Lower reproduction rates and production of offspring are a common phenomenon in cases where the ewes had lower live weights and body fat at the commencement of mating (Ptacek *et al.*, 2014). Ptacek (2014) found a positive correlation between an increase in lamb weight and the live weight of the ewe at mating. Abdel-Mangeed and Abo el-Maaty (2012) found that lamb live weight at birth and weaning were higher for the lambs whose mothers had higher body condition scores.

2.8.5 Season

Seasonality of oestrus is controlled by the hours of light to which the ewe is exposed. Generally speaking is the main mating seasons in South Africa during autumn. Autumn pairing coincides with the peak of sexual activity in woolled sheep of the southern hemisphere. In the Western Cape are ewes paired in spring to ensure good fodder for ewe recovery after lambing in autumn and high pasture covers for lambs survival in winter, seeing as the Western Cape is a winter rainfall area.

Sheep naturally breed at times of the year when night-time is longer and daytime is shorter, hence the term short-day breeders. In temperate areas of the world, ewes tend to enter a non-reproductive state during the spring and summer and start cycling in the fall as the day length decreases.

As the gestation length for the ewe is ~5 months long, fall breeding means the lambs are born in the spring. Under natural conditions, the warmer weather and ample feed during spring make it a favourable time of the year for lamb survival and growth. This fact means that the season of breading can be detrimental to sheep farmers in South Africa, seeing that majority of the national sheep herd are found in the Northern Cape, Free State and the Eastern Cape. In these areas in South Africa (specially for most of the last decade) is higher DM availability in the forage not appreciated as abundance of food for the sheep, but as an opportunity for the sheep to catch up body condition that were lost during winter.

Merino, Dorset Horn and Rambouillet are breeds that developed in temperate climates. They are typical examples of animals that express oestrus and anoestrus at different times of the year (Dardente *et al.*, 2016).

2.8.6 Heat stress

Heat stress is known to have a negative effect on male fertility as well as certain female productive traits (Marai *et al.*, 2007). Incidences of longer oestrous cycles, with lower ovulation rates, have been found to be a consequence of high environmental temperatures (Dias e Silva *et al.*, 2016). Heat stress can interfere with the expression of normal mating behaviour, which in turn will negatively impact on interaction between rams and ewes, which will ultimately result in lower lambing rates (Setchell, 2014).

2.9 The purpose of the study

The purpose of the study was therefore to evaluate one such a “super-fertility” diet with a commercially available flushing diet, and a simple flushing diet consisting only of sweet lupins (*Lupinus angustifolius*) in terms of their effect of ewe live weight, back-fat thickness, number of follicles, average follicle size, number of pre-ovulatory follicles, and number of lambs born. This will enable the calculation of whether the feeding of expensive “super-fertility” diets justify the input costs associated with it.

Chapter 3: Methodologies

3.1 Ethical approval details

Ethical approval was obtained from the Animal Care and Use Ethics Committee of the University of Stellenbosch (ACU Reference #: ACU-2019-6629).

3.2 Experimental location

The study was conducted between 18 September and 31 October 2019 on the Mariendahl Experimental Farm of Stellenbosch University, which is located about 14 km outside Stellenbosch in the Western Cape Province (latitude:-33.843947; longitude: 18.817218). Stellenbosch lies on 128m above sea level, and the area is characterized by a warm, temperate climate. The climate in the region is classified as Csb by the Köppen-Geiger system. The temperature here averages 16.4 °C | 61.5 °F. The annual rainfall, of which most is received during the winter season, is 802 mm.

3.3 Experimental animals and husbandry

Forty-eight Dohne Merino ewes, aged between 2 and 6 years of age, were used in the study. The live weight of the ewes ranged between 58kg and 95kg, and the average body condition score ranged from 2.5 to 3 prior to the feeding of the flushing diets (Suiter, 1994).

The ewes originated from one resource flock and were managed as a single mating group throughout the trial. This adhered to the management practice on the farm, where a mating group consist of 50 ewes is allocated to one ram. Before the commencement of the trial, the ewes were maintained in one camp, and were visually and physically isolated from the breeding rams.

The teaser ram was introduced 13 days prior to the introduction of the intact ram, as part of the behavioural stimulus of the teaser ram effect, used in combination with a flushing diet to synchronize oestrus in the ewes (Gootwine, 2016).

The experimental animals were monitored daily for general wellbeing. Any signs of abnormal behaviour, respiratory abnormality, and any other abnormal distress behaviour were recorded, and addressed. No intervention was required throughout the study.

3.4 Experimental design and treatments

The treatment diets consisted of sweet lupins (L), a commercially available standard flushing diet (SFD), a commercially available super-fertility diet (SUFD), and a control diet (no flushing diet provided; C). The experimental animals were allocated to the treatment groups to ensure that all age groups were represented in the respective treatment groups, and identified with ear-tags that differed in terms of colour to allow for the easy separation of the ewes for weighing, feeding, and scanning purposes.

The composition of the respective treatment diets is presented in Table 3.1.

Table 3.1. The composition of the different diets that was fed to Dohne Merino ewes to assess the influence of ovarian dynamics, implantation and lambing rate.

Diet component	No flushing	Standard flushing diet (control)	Super embryo diet	Lupins diet
Moisture (%)	0	11.5	11.47	10.0
Protein (%)	0	19.44	14.43	37.0
Fat (%)	0	3.32	4.76	8.9
Fibre (%)	0	6.24	11.76	10.7
Ash (%)	0	3.58	8.29	3.0
Starch (%)	0	32.45	21.67	2.1
NPN Protein (%)	0	31.9	8.17	0
UDP (%RP)	0	27.0	29.18	14.0
Urea (%)	0	1.88	0.29	0
Ca (%)	0	0.94	1.26	0.3
P (%)	0	0.48	0.48	0.45
ME (MJ/kg)	0	10.6	9.8	12.8

The experimental diets were fed to the ewes for six weeks, i.e. for a period of three weeks before the introduction of the intact ram, up and to three weeks after the introduction of the intact ram. Each ewe received 250g/ewe/day, and feeding was carried out in the morning, after completion of blood collection and ultrasound scanning procedures. Any feed refusals were recorded. All feed was consumed, and therefore results on this will not be presented.

The teaser ram was introduced to the experimental flock on 25 September 2019, one week after the start of the feeding of the experimental diets. The teaser ram was maintained with

the ewes for a period of 13 days, after which the teaser ram was replaced with an intact ram. The intact ram was maintained with the ewes for a period of 30 days, after which the intact ram was removed and added to the breeding ram flock maintained on the same farm.

Pregnancy diagnosis was performed by an experienced veterinary technician from the Overberg Animal Hospital 40 days after the removal of the intact ram (A. García et al., 1993). Based on the ultrasound findings, ewes were then separated into flocks of ewes carrying single foetuses or twins/triplets to allow for the appropriate nutritional management of the ewes.

Table 3.2 presents the timeline of specific actions carried out during the trial.

Table 3.2. Timeline of the trial to indicate the intervals at which data recording were carried out.

Date	Action
2019/09/18	Group 3 (SUFD) Treatment starts, weighing of ewes commence, scan back-fat thickness all animals
2019/09/19	Group 1 (L) and 2 (SFD)Treatment start
2019/09/25	Teaser ram in/Follicular scanning protocol begins for group 3 (SUFD) and 4 (Control)
2019/09/26	Follicular scanning protocol begins for group 1 (L) and 2 (SFD)
2019/10/08	Intact ram in / Teaser ram out
2019/10/09	Scan back-fat thickness of all ewes
2019/10/11	2nd last day of weighing (no weighing 12/10- 17/10)
2019/10/18	Weigh all ewes the last time
2019/10/30	Last day of flushing group 3 (SUFD), scan back-fat thickness all animals.
2019/10/31	Last day of flushing group 1 (L) and 2 (SFD)
2019/11/08	Intact ram out
2020/01/07	Pregnancy diagnosis (60 days after intact ram is removed)

3.5 Proximate analysis of the experimental diets

Proximate analysis was conducted to determine the percentage moisture, ash, crude protein, crude fibre, and crude fat in the treatment diets, i.e. sweet lupins, standard fertility diet, and the super-fertility diet.

3.5.1 Moisture determination

The AOAC Official Method 934.01 (AOAC, 2002) was used for moisture analysis. Approximately 2 g from samples were weighed out and placed into porcelain crucibles and

dried for 24 h at 100°C in a forced air-drying oven. The percentage moisture was calculated using the following formula:

Moisture (%) = [(wet weight (g) – dry weight (g)) / wet weight (g)] x 100.

3.5.2 Ash determination

Ash content was determined using the AOAC Official Method 942.05 (2002). Approximately 2.0 g of sample was placed into a dry, pre-weighed crucible and then inserted into a furnace at 500°C for 6 hours. The percentage ash was calculated using the following formula:

Ash (%) = (ash weight (g)/ wet weight (g)) x 100.

3.5.3 Crude protein determination

Crude protein content was determined as described by the AOAC Official Method 992.15 (2002), in which a nitrogen determiner was used. Percent protein was calculated by multiplying total percentage nitrogen by a protein conversion factor of 6.25.

Crude protein from food, plant material and other products is measured by its nitrogen content, including both true protein and non-protein nitrogen. The Dumas method with a LECO FP 528 was used for the quantitative determination of nitrogen in the samples.

Percentage crude protein was calculated using the following formula:

Crude protein (%) = % Nitrogen X 6.25.

3.5.4 Crude fibre determination

Crude fibre is determined gravimetrically, after chemical digestion and solubilisation of other compounds present (i.e. protein, starch, and other digestible/solubilisable carbohydrates) with diluted sulphuric acid and sodium hydroxide. Briefly, the samples are first boiled in diluted acid and then in diluted alkali. The acid hydrolysis removes free sugars and starch. The alkaline hydrolysis removes protein and some carbohydrates.

The fibre mass is then corrected for ash after ignition, and crude fibre percentage is calculated using the following formula:

Crude fibre (%) = [(A-B)/Sample mass (g)]X100

where A = Mass of residue (in grams) in crucible after drying, and B = Mass of residue (in grams) in crucible after ashing.

3.5.5 Crude fat determination

The AOAC Official Method 954.02 (2000) was used to determine the crude fat content.

Crude fat is determined by acid hydrolysis with HCl followed by extraction of hydrolysed lipid materials with mixed ethers. The ether is then evaporated, and the residue is heated to a constant weight and expressed as percentage crude fat.

Percentage fat is calculated using the following formula:
Fat (%) = [(Mass of Soxhlet cup+ fat) - (mass of Soxhlet cup)]/Mass of sample (gram)X100.

3.5.6 Live weight

Ewes were weighed daily from the beginning of the trial until three days after the introduction of the intact ram, using a Rudd scale (model W210). Weighing was performed between 06:30 and 07:00. Handling of the ewes was minimized after this period to avoid disruption of normal mating behaviour and activity (Braden and Moule, 1964; Sawyer *et al.*, 1979a, b). Ewes were weighed towards the end of the trial (i.e. on 18 October 2019) to obtain an end weight for regression analysis of ADG.

3.5.7 Ultrasound measurement of back-fat thickness

The back-fat thickness was determined using diagnostic imaging, using a Mindray DP-30 veterinary ultrasound scanner fitted with a 75L53EA transducer (5 MHz linear array probe), as described by Abdel-Mageed and Abo El Maaty (2012). Back-fat thickness was recorded at three time points during the trials, i.e. at the beginning of the trial (18/09/2019), the day after the intact ram was introduced (09/10/2019), and three weeks after the intact ram was introduced (30/10/2019). Back-fat thickness was measured in millimetres.

3.5.8 Ultrasound evaluation of ovarian activity

Ovarian ultrasonography is a useful tool in tracking the progression of antral follicular development, and presence of pre-ovulatory follicles, as well as the formation of the corpus luteum (Schrick *et al.*, 1993; Gonzalez-Bulnes *et al.*, 2000; Duggavathi *et al.*, 2003a, b; Vi˜noles *et al.*, 2004).

Ewes were scanned daily by the same operator, using transrectal ultrasonography to assess degree of follicular development on the ovaries. Transrectal ultrasonographic examinations were performed using a Mindray DP-30 veterinary ultrasound scanner fitted with a 75L50EAV transducer (7.5 MHz linear array probe).

Scanning commenced 7 days after the onset of feeding of the treatment diets, and continued for a total of 16 days (Toosi *et al.*, 2009). Settings (near-field, far-field and overall gain) were

optimised prior to the beginning of the trail and used throughout the study. A visual presentation of the ultrasound scanning with ewes in standing position are portrayed in Figure 3.1.

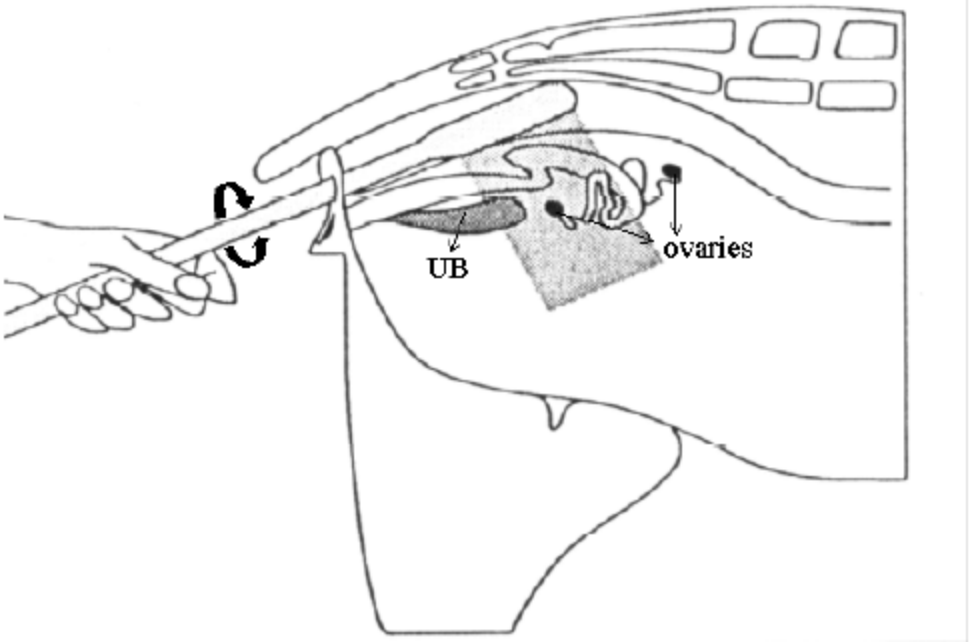

Figure 3.1. Transrectal ultrasonographic examination of the reproductive tract with the ewe in standing position. In cranial position to the urinary bladder (UB), the transducer is moved clockwise and anti-clockwise to locate the ovaries (adapted from Kähn, 1994).

The number and diameter of all follicles visible on both ovaries were recorded daily. Follicle size was measured in millimetres to allow for the classification of the follicles, as described by Sousa (2011). Analysis of follicle data required the classification of follicles into the size classes of small, medium, large, and ovulatory, as described by (Gonzalez-Bulnes and Veiga-Lopez, 2005).

During the study the smallest follicles identifiable was 1.354mm and follicles are considered to be in an antral stage where they are <2.2mm in size (Gil, 2003). Therefore follicles <3mm were classified as small. The follicles that had a diameter that ranged from 3mm to 5mm were categorized as medium follicles. The pre-ovulatory size (i.e. a minimum of 4 mm in diameter) is typically attained about four days later (Turnbull *et al.*, 1977). The follicles that had a diameter that ranged between 5mm and 6mm, were categorized as large follicles. The ovulary follicles were identified as follicles that had a minimum diameter of 6mm.

The analyses on ovulatory follicles could only have been done from the 16th day that the ewes have been in the trial seeing that only from there all groups had ovulatory follicles present and visible. For this normal pattern of follicular recruitment and subsequent development to be realized, and to allow a ewe the ability to perform according to her genetic breeding potential, it becomes even more imperative that the nutritional influence of a flushing diet on follicular development in the ewe is properly understood.

3.5.9 Detection of heat

Prior to the introduction of the teaser ram, ewes were physically and visually isolated for a minimum period of six weeks from the breeding rams on the farm. Signs of oestrus in the ewe are much less pronounced than in the cow or doe and can usually not be detected unless a ram is present.

A vasectomised ram was used as a teaser ram and was introduced to the ewes on 25 September 2019 to stimulate oestrus and also to allow for detection of oestrus ewes. On 8 October 2019, the teaser ram was removed from the ewes, and an intact ram was introduced. A marking medium comprising of butter and ochre was applied on the chest of the intact ram, which facilitated the identification of ewes that were jumped by the ram. Ewes were assumed to be in heat when she was spotted with a greasy brown stain on her hind quarter.

3.5.10 Lambing

Ewes lambed during the February-March 2020 lambing season. The birth weight of each lamb was recorded on the day it was born.

Lambing percentage (LP) is calculated by the following formula:

LP = (Number of lambs born alive/Number of ewes exposed to ram) x 100.

3.6 Statistical analysis

3.6.1 Chapter 4

Statistical analyses were performed using Microsoft Excel Stat 2020 with modules XLSTAT-Premium. To determine the ADG of each individual ewe over time, a linear regression of ewe live weight over time was calculated. The slope of the regression equation represented the ADG. In order to correct for the non-homogeneous initial live weights of the ewes, the weight intercept of the linear regression calculated for each ewe was used as a co-variant in an ANCOVA model to determine if the accumulated ADG was influenced by the treatment diet, or influenced by ewe age.

Though the distribution of the ewes into the various treatment groups was homogeneous according to age, there were differences in initial back-fat thickness measured. The back-fat thickness analyses were approached as follows, i.e. firstly, an ANOVA was performed, using the difference between the back-fat thickness measured on 18/09/2019 and 09/10/2019, and between the back-fat thickness of the ewes on 18/09/2019 and 30/10/2019. In this model the aim was to determine if the difference in back-fat thickness was influenced by treatment diets and/or ewe age. With the second approach, an ANCOVA analysis was carried out to determine if the treatment diets and/or ewe ages differed significantly in order to explain the change in back-fat thickness of the ewes, where the back-fat thickness at the beginning of the trial and the back-fat thickness of the ewes on 30 October were used as co-variants. Age was also added in the model to determine if the age difference significantly contributed to the change in back-fat thickness throughout the trial.

An ANOVA was performed to determine the influence of ewe age and treatment diet on mean follicle size. The influence of the live weight of the ewes on the mean follicle size was determined through a Pearson pairwise correlation test. The correlation test was performed with the mean weights of the ewes and the mean follicle sizes within the ewes on exactly the same dates. The correlation between the average ewe live weight and average follicle size represent an estimation of the “static effect” of flushing (Torre *et al.*, 1991).

3.6.1 Chapter 5

Statistical analyses were performed using Microsoft Excel Stat 2020 with modules XLSTAT-Premium. An ANOVA was performed to determine the influence of ewe age and treatment diet on the total follicle volume. An ANOVA was performed using the slope of the average follicle size to determine the average daily gain in follicle size over time. To accommodate the difference in follicle size at the beginning of the ultrasound period, an ANCOVA was performed using the slope of the average follicle size, with the intercept of the slope as a co-variant.

An ANOVA was carried out to determine the influence of treatment diet and ewe age on the number of small, medium, large and ovulatory follicles.

A Chi-square test was carried out on the number of lambs born to determine if treatment diet influenced the number of lambs born. An ANOVA analysis was also performed to determine whether treatment diet and/or ewe age had an influence on lamb birth weight.

Chapter 4: The influence of flushing diet composition on live weight, back-fat thickness and follicular development in Dohne Merino (*Ovis aries*) ewes

4.1 Abstract

The study investigated the influence of different flushing diets on the live weight, back-fat thickness, and follicle development in semi-intensively managed Dohne-Merino ewes. The different flushing diet treatments consisted of a flushing diet consisting of sweet lupins (L) only, a commercially available industry-standard flushing (SFD) diet, and a commercially available super-fertility flushing diet (SUFD). A total of 48 sexually mature Dohne Merino ewes were allocated to four treatment groups, including to the control group that received no flushing diet. The flushing diets were fed at 250g/day/ewe for 6 weeks, i.e. three weeks prior to the introduction of the intact ram until three weeks after the rams was introduced. Parameters recorded include live weight, back-fat thickness (BT), number of follicles observed on ovaries, and mean follicle size. Treatment diet significantly influenced the ADG of the ewes and accumulated in a dynamic effect of flushing, where SFD-fed ewes gained 0.125g/day (SE ± 0.018), followed by SUFD-fed ewes (0.122g/day, SE ± 0.023), L-fed ewes (0.098g/day, SE ± 0.024), and the control group ewes (0.015g/day, SE ± 0.026). The change in BT measured at the end and beginning of the trial, respectively, was significantly influenced by ewe age, with the biggest difference in BT for ewes aged three years (0.316mm, SE ± 0.025), followed by ewes aged four years (0.198mm, SE ± 0.030), ewes aged six years (0.063mm, SE ± 0.030), ewes aged two years (0.034, SE ± 0.017), and the ewes aged five years that lost backfat (-0.011mm, SE ± 0.038). Ewe age significantly contributed to the variation in follicle size, with average follicle size the largest for ewes aged two (SE ± 0.079)- and three years respectively (4.412mm, SE ± 0.101), followed by ewes aged six years (4.408mm, SE ± 0.092), ewes aged four years (4.271mm, SE ± 0.085), and the ewes aged five years had the smallest average follicle size (4.097mm, SE ± 0.094). The interaction between treatment and age was significant for SUFD-fed ewes 5 years of age, and SFD-fed ewes 6 years of age. No correlation was observed between ewe live weight and mean follicle size.

4.2 Introduction

Various studies report on the influence of latitude and climate on the reproductive strategy of sheep breeds, with sheep breeds originating from temperate climates at mid or high latitudes adopting a seasonal breeding strategy, with photoperiod being the determining *zeitgeber* in regulating reproductive activities (Gonzalez-Bulnes *et al.*, 2003; Letelier *et al.*, 2009; Smith and Clarke, 2010). Sheep breeds being farmed with in tropical and sub-tropical environments adopt an aseasonal or intermittent poly-oestrus breeding season, and in this case, the quality and availability of food will determine the onset of breeding activity (Gonzalez-Bulnes *et al.*, 2003; Rosa and Bryant, 2003).

The productivity and profitability of local meat and milk industries effectively depend on reproductive performance (Martin *et al.*, 2004). The level of nutrition and quality of feed provided is one of the most important factors that influences the reproductive performance of ruminants, especially small ruminants. The provision of flush feeding is a practice that is

commonly used in the sheep industry to improve the overall reproductive performance of the flock, in combination with the ram effect (Gootwine, 2016). The practice of flush feeding involves the feeding of diets that are higher in protein and energy to ewes prior to the introduction of the intact ram, as well as during the mating period, for a minimum of two weeks. Prior to the feeding of a flushing diet, ewes will be maintained on a maintenance diet that serves the purpose to meet their daily nutritional need to support maintenance functions. The provision of excess protein and energy, i.e. more than what is required for maintenance, allows an ewe to partition the excess energy and protein towards physiological taxing processes such as folliculogenesis, fertilization, conception, gestation, parturition and lactation (Cárdenas *et al.*, 1997; Michels *et al.*, 2000 Delgadillo *et al.*, 2009; Fitzgerald *et al.*, 2015; Habibizad *et al.*, 2015; 2018; Nogueira *et al.*, 2015; Shad *et al.*, 2016; Mpebe *et al.*, 2018).

The ultimate effect of nutrition on folliculogenesis, ovulation rate, fertilization, gestation, and ultimately weaning success, depends on the net nutritional status of the ewe, which in turn is influenced and determined by endogenous and exogenous sources of nutrients available to the ewe (Downing and Scaramuzzi, 1991). There is a direct link between ewe body condition and ovulation rate, with terms such as static, dynamic, and immediate that can be used to describe the nutritional effect of flush feeding (Coop, 1966; Toosi *et al.*, 2009). The term “static effect” refers to the higher ovulation rate observed in heavy compared to lighter ewes, while the “dynamic effect” refers to increases in ovulation rate due to an increase in live weight and improvement in body condition during short periods, such as the three weeks of supplementation before the introduction of an intact ram (Smith and Stewart, 1990).

Ovulation rate is influenced by several factors, of which nutrition is considered as the most important factor. The effect of nutrition on ewe ovulation rate is evident over time and can thus be considered as part of a nutritional continuum, measured at different times relative to the commencement of feeding (Smith and Stewart, 1990). Underwood and Shier (1941) stated that flush feeding impacts positively on the number, maturation, and ovulation rate of ova during an oestrous cycle. Ocak *et al.* (2006) found that short-term supplementation (i.e. 15-17 days) post-mating can beneficially influence the non-return rate and lambing rate after the first oestrus, as well as litter size in ewes. When the composition of the flushing diet is considered, it is important that supplementation with the flushing diet results in a significant weight gain in ewes that will impact positively on the ovulation and conception rate, and ultimately weaning success (Marshall and Potts, 1924; Darlow and Hawkins, 1933; Clark, 1934; Friedman and Turner, 1939).

Several studies stated that fodder of good quality, whether it be pastures of grain, and that is considered palatable by the animal, will have the desired effect in ewes receiving such supplemental flush feeding (Bray, 1925; Geary, 1956). Several studies reported on the inclusion of lupins (*Lupinus angustifolius*) in a supplement ration before mating, and the positive influence on the reproduction rate of sheep (Nottle *et al.*, 1997). A period of four to six days of supplementation with lupins were found to improve ovulation rate of ewes, with no changes in live weight or body condition (Knight *et al*., 1975; Lindsay, 1976; Oldham and Lindsay, 1984; Stewart and Oldham, 1986; Downing *et al*., 1995b). This effect has been called the “immediate nutrient effect”.

Feeding supplemental fatty acids provides a higher level of energy and have positive impacts on reproductive performance by controlling ovarian function and follicular development (Gulliver *et al.*, 2012a; Nieto *et al.*, 2015). Different types of lipids can have different impacts on animal performance and reproductive functions due to different fatty acid profiles. Poly-unsaturated FA (PUFAs) are a group of fats containing 16 to 22 carbon atoms and more than one double bond. In ruminants, feeding dietary omega-6 (n-6) or omega-3 (n-3) PUFA has led to several positive effects on female reproductive success (Gulliver *et al.*, 2012a). Lactating dairy cows fed with different sources of PUFAs showed an increased number and size of pre-ovulatory follicles (Nieto *et al.*, 2015). Omega-3 polyunsaturated fatty acids (n-3) are known to have a positive effect on human and animal health. In particular, the ratio of omega-6 polyunsaturated fatty acids (n-6) to n-3 may play an important role in several aspects of animal health, production, and reproduction (Abayasekara and Wathes, 1999). The primary fatty acids of interest in studies investigating reproduction in animals are the long-chain n-3 including eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3), and the long-chain n-6 arachidonic acid (AA, 20:4n-3). These long-chain PUFAs are synthesised in the body from the short-chain n-3 linolenic acid (ALA, 18:3n-3) and n-6 linoleic acid (LA, C18:2n-6) through a number of steps involving desaturation and elongation. The short-chain ALA and LA cannot be synthesised by animals, and therefore need to be supplemented in the diet (Lands, 1992).

While the conversion of ALA and LA to long-chain PUFA is rate-limited (Emken *et al.*, 1994; Pawlosky *et al.*, 2001), there is considerable evidence that significant metabolism occurs in ruminants and concentrations of n-3 in plasma (Kemp *et al.*, 1998), red blood cells (Gulliver *et al.*, 2010), meat (Scollan *et al.*, 2006), milk (Dewhurst *et al.*, 2003) and reproductive tissue (Kim *et al.*, 2001) are influenced by concentrations of ALA and LA in the diet. There are several sources of the short-chain n-3 ALA in ruminant diets, including forages and linseed. Long chain n-3 (20 carbons or more) purified from sources such as fish oil and fishmeal can also

be fed to ruminants, and these are usually protected against rumen biohydrogenation (Ashes *et al.*, 1992). These PUFA supplements are usually expensive; however, it may potentially result in higher profits when the improvement in number of lambs weaned is considered.

Diagnostic imaging presents a non-invasive way to measure back-fat (Esquivelzeta *et al.*, 2012) as well as study ovarian dynamics during an oestrus cycle (Ślósarz *et al.*, 2003). The use of body condition scoring (BCS) of ewes using a five-point scoring system, where a score of 1 indicates an emaciated ewe, and a score of 5 an obese ewe, can provide a relative but subjective indication of the nutrition status of an ewe prior to the flushing period (Kenyon *et al.*, 2014). Abdel-Mageed and El-Maaty (2012) suggested in an effort to overcome the limitation of BCS, the ultrasound measurement of back-fat thickness in the area of the end part of the thoracis vertebra as an alternative method for BCS. The use of ultrasound to evaluate ovarian follicular development, ovulation, and the stage at which the corpus luteum (CL) forms, has been reported by many authors as a useful tool in reproductive studies (Turnbull *et al.*, 1977; Cahill and Mauleon, 1980; Schrick *et al.*, 1993; Gonzalez-Bulnes *et al.*, 2000; Duggavathi *et al.*, 2003a, b; Vi˜noles *et al.*, 2004).

In the South African animal feed industry, certain animal feed companies formulate diets that are referred to as “super-fertility” diets, which are considerably more expensive than standard flushing diets. The assumption that the feeding of such “super-fertility” diets will result in an improvement of weaning percentage of flocks through having a beneficial effect on follicular development and subsequent conception rates of ewes, which in turn will decrease input costs for the sheep producer. No studies have however been conducted to verify these assumptions. The purpose of this study was therefore to compare a commercially available “super-fertility” diet, a commercially available industry-standard flushing diet, and a flushing diet consisting of lupins only (the latter which some producers feed to their ewes prior to the mating season), in terms of the effect on average daily gain, back-fat thickness, and ovarian activity in sexually mature Dohne Merino ewes. Findings form this study will assist in determining whether the resulting lamb weaning weight and weaning success are justified by the input costs associated with each respective diet.

4.3 MATERIALS AND METHODS

Ethical approval was obtained from the Animal Care and Use Ethics Committee of the University of Stellenbosch (ACU Reference #: ACU-2019-6629).

4.3.1 Experimental location

The study was conducted between 18 September and 31 October 2019 on the Mariendahl Experimental Farm of Stellenbosch University, which is located about 14 km outside Stellenbosch in the Western Cape (latitude:-33.843947; longitude: 18.817218). Stellenbosch lies on 128m above sea level, and the area is characterized by a warm, temperate climate. The climate in the region is classified as Csb by the Köppen-Geiger system. The temperature here averages 16.4 °C | 61.5 °F. The annual rainfall, of which most is received during the winter season, is 802 mm.

4.3.2 Experimental animals and husbandry

Forty-eight Dohne Merino ewes, aged between 2 and 6 years of age, were used in the study. The live weight of the ewes ranged between 58kg and 95kg, and the average body condition score ranged from 2.5 to 3 prior to the feeding of the flushing diets (Suiter, 1994).

The ewes originated from one resource flock and were managed as a single mating group throughout the trial. This adhered to the management practice on the farm, where a mating group consist of 50 ewes is allocated to one ram. Before the commencement of the trial, the ewes were maintained in one camp, and were visually and physically isolated from the breeding rams.

The teaser ram was introduced 13 days prior to the introduction of the intact ram, as part of the behavioural stimulus of the teaser ram effect, used in combination with a flushing diet to synchronize oestrus in the ewes (Gootwine, 2016).

The experimental animals were monitored daily for general wellbeing. Any signs of abnormal behaviour, respiratory abnormality, and any other abnormal distress behaviour were recorded, and addressed. No intervention was required throughout the study.

4.3.3 Experimental design and treatments

The treatment diets consisted of lupins (Treatment 1; L), a commercially available flushing diet (Treatment 2; SFD), a commercially available super-fertility diet (Treatment 3; SUFD), and a control diet (no flushing diet provided; C). The experimental animals were allocated to the treatment groups to ensure that all age groups were represented in the respective treatment groups, and identified with ear-tags that differed in terms of colour to allow for the easy separation of the ewes for weighing, feeding, and scanning purposes.

The composition of the respective treatment diets is presented in Table 4.1.

Table 4.1. The nutritional composition of the treatment diets fed to Dohne Merino ewes prior to and during the 2019 breeding season.

Diet component	No flushing (Control)	Standard flushing diet	Super embryo diet	Lupins diet
Moisture (%)	0	11.5	11.47	10.0
Protein (%)	0	19.44	14.43	37.0
Fat (%)	0	3.32	4.76	8.9
Fibre (%)	0	6.24	11.76	10.7
Ash (%)	0	3.58	8.29	3.0
Starch (%)	0	32.45	21.67	2.1
NPN Protein (%)	0	31.9	8.17	0
UDP (%RP)	0	27.0	29.18	14.0
Urea (%)	0	1.88	0.29	0
Ca (%)	0	0.94	1.26	0.3
P (%)	0	0.48	0.48	0.45
ME (MJ/kg)	0	10.6	9.8	12.8

The experimental diets were fed to the ewes for six weeks, i.e. for a period of three weeks before the introduction of the intact ram, up and to three weeks after the introduction of the intact ram. Each ewe received 250g/ewe/day, and feeding was carried out in the morning, after completion of blood collection and ultrasound scanning procedures. Any feed refusals were recorded. All feed was consumed, and therefore results on this will not be presented.

The teaser ram was introduced to the experimental flock on 25 September 2019, one week after the start of the feeding of the experimental diets. The teaser ram was maintained with the ewes for a period of 13 days, after which the teaser ram was replaced with an intact ram. The intact ram was maintained with the ewes for a period of 30 days, after which the intact ram was removed and added to the breeding ram flock maintained on the same farm.

Pregnancy diagnosis was performed by an experienced veterinarian technician from the Overberg Animal Hospital 40 days after the removal of the intact ram (A. García et al., 1993). Based on the ultrasound findings, ewes were then separated into flocks of ewes carrying single foetuses, and twins/triplets to allow for the appropriate nutritional management of the ewes. Table 4.2 presents the timeline of specific actions carried out during the trial.

Table 4.2. Timeline of the trial to indicate the intervals at which data recording were carried out.

Date	Action
2019/09/18	SUFD treatment starts, all animal weighing starts, scan back-fat thickness all animals
2019/09/19	L and SFD treatment start
2019/09/25	Teaser ram in/ Follicular scanning protocol begins for SUFD and Control groups
2019/09/26	Follicular scanning protocol begins for L and SFD groups
2019/10/08	Intact ram in/ Teaser ram removed
2019/10/09	Scan back-fat thickness all animals
2019/10/11	2nd last day of weighing (no weighing 12/10- 17/10)
2019/10/18	Weigh all animal one last time
2019/10/30	Last day of flushing fed to SUFD group, scan back-fat thickness all animals.
2019/10/31	Last day of flushing diet fed to L and SFD groups
2019/11/08	Intact ram out
2020/01/07	Pregnancy scan (60 days after ram out)

4.3.4 Proximate analysis of the experimental diets

Proximate analysis was conducted to determine the percentage moisture, ash, crude protein, crude fibre, and crude fat in the treatment diets, i.e. sweet lupins, standard fertility diet, and the super-fertility diet.

4.3.4.1 Moisture determination

The AOAC Official Method 934.01 (AOAC, 2002) was used for moisture analysis. Approximately 2 g from samples were weighed out and placed into porcelain crucibles and dried for 24 h at 100°C in a forced air-drying oven. The percentage moisture was calculated using the following formula:

$$\text{Moisture (\%)} = [(\text{wet weight (g)} - \text{dry weight (g)}) / \text{wet weight (g)}] \times 100.$$

4.3.4.2 Ash determination

Ash content was determined using the AOAC Official Method 942.05 (2002). Approximately 2.0 g of sample was placed into a dry, pre-weighed crucible and then inserted into a furnace at 500°C for 6 hours. The percentage ash was calculated using the following formula:

Ash (%) = (ash weight (g)/ wet weight (g)) x 100.

4.3.4.3 Crude protein determination

Crude protein content was determined as described by the AOAC Official Method 992.15 (2002), in which a nitrogen determiner was used. Percent protein was calculated by multiplying total percentage nitrogen by a protein conversion factor of 6.25.

Crude protein from food, plant material and other products is measured by its nitrogen content, including both true protein and non-protein nitrogen. The Dumas method with a LECO FP 528 was used for the quantitative determination of nitrogen in the samples.

Percentage crude protein was calculated using the following formula:

Crude protein (%) = % Nitrogen X 6.25.

4.3.4.4 Crude fibre determination

Crude fibre is determined gravimetrically, after chemical digestion and solubilisation of other compounds present (i.e. protein, starch, and other digestible/solubilisable carbohydrates) with diluted sulphuric acid and sodium hydroxide. Briefly, the samples are first boiled in diluted acid and then in diluted alkali. The acid hydrolysis removes free sugars and starch. The alkaline hydrolysis removes protein and some carbohydrates.

The fibre mass is then corrected for ash after ignition, and crude fibre percentage is calculated using the following formula:

Crude fibre (%) = [(A-B)/Sample mass (g)]X100

where A = Mass of residue (in grams) in crucible after drying, and B = Mass of residue (in grams) in crucible after ashing.

4.3.4.5 Crude fat determination

The AOAC Official Method 954.02 (2000) was used to determine the crude fat content. Crude fat is determined by acid hydrolysis with HCl followed by extraction of hydrolysed lipid

materials with mixed ethers. The ether is then evaporated, and the residue is heated to a constant weight and expressed as percentage crude fat.

Percentage fat is calculated using the following formula:
Fat (%) = [(Mass of Soxhlet cup+ fat) - (mass of Soxhlet cup)]/Mass of sample (gram)X100.

4.4 Data recorded

4.4.1 Live weight

Ewes were weighed daily from the beginning of the trial until three days after the introduction of the intact ram, using a Rudd scale (model W210). Weighing was performed between 06:30 and 07:00. Handling of the ewes was minimized after this period to avoid disruption of normal mating behaviour and activity (Braden and Moule, 1964; Sawyer *et al.*, 1979a, b). Ewes were weighed towards the end of the trial (i.e. on 18 October 2019) to obtain an end weight for regression analysis of ADG.

4.4.2 Ultrasound measurement of back-fat thickness

The back-fat thickness in the ewes were determined using diagnostic imaging, using a Mindray DP-30 veterinary ultrasound scanner fitted with a 75L53EA transducer (5 MHz linear array probe), as described by Abdel-Mageed and Abo El Maaty (2012). Back-fat thickness was recorded at three time points during the trials, i.e. at the beginning of the trial (18/09/2019), the day after the intact ram was introduced (09/10/2019), and three weeks after the intact ram was introduced (30/10/2019). Back-fat thickness was measured in millimetres.

4.4.3 Ultrasound evaluation of ovarian activity

Ewes were scanned daily by the same operator, using transrectal ultrasonography to assess degree of follicular development on the ovaries. Transrectal ultrasonographic examinations were performed using a Mindray DP-30 veterinary ultrasound scanner fitted with a 75L50EAV transducer (7.5 MHz linear array probe).

Scanning commenced 7 days after the onset of feeding of the nutritional diets and continued for a total of 16 days (Toosi *et al.*, 2009). Settings (near-field, far-field and overall gain) were optimised prior to the beginning of the trail and used throughout the study. The number and diameter of all follicles visible on both ovaries were recorded daily. Follicle size was measured in millimetres to allow for the classification of the follicles, as described by Sousa (2011).

4.5 Statistical analyses

Statistical analyses were performed using Microsoft Excel Stat 2020 with modules XLSTAT-Premium. To have determined the ADG of each individual ewe over time, a linear regression of ewe live weight over time was calculated. The slope of the regression equation represented the ADG. In order to have corrected for the non-homogeneous initial live weights of the ewes, the weight intercept of each linear regression of each ewe was used as a co-variant in an ANCOVA model to determine if the accumulated ADG was influenced by the treatment diet fed or the ages of the ewes.

Though the distribution of the ewes into the various treatment groups was homogeneous according to age, there were differences in initial back-fat thickness measured. The back-fat thickness analyses were approached as follows, i.e. firstly, an ANOVA was performed, using the difference between the back-fat thickness measured on 18/09/2019 and 09/10/2019, and between the back-fat thickness of the ewes on 18/09/2019 and 30/10/2019. In this model the aim was to determine if the difference in back-fat thickness was influenced by treatment diets.

With the second approach, an ANCOVA analysis was carried out to determine if the treatment diets differed significantly in order to explain the change in back-fat thickness of the ewes, where the back-fat thickness at the beginning of the trial and the back-fat thickness of the ewes on 30 October were used as co-variants. Age was also added in the model to determine if the age difference significantly contributed to the change in back-fat thickness throughout the trial.

An ANOVA was performed to determine the influence of ewe age and treatment diet on mean follicle size. The influence of the live weight of the ewes on the mean follicle size was determined through a pairwise correlation test.

4.6 RESULTS

4.6.1 Proximate analysis of the treatment diets

Table 4.3 presents the proximate analysis results of the percentage moisture, percentage ash, percentage crude fat, percentage crude fibre, and percentage crude protein determined for the respective treatment diets.

Table 4.3. Proximate analysis results of the treatment diets fed to sexually mature Dohne Merino ewes for a period of six weeks during the natural mating season of 2019.

Treatment diet	Moisture (%)	Ash (%)	Crude fat (%)	Crude fibre (%)	Crude protein (%)
Sweet lupins	9.23	3.16	5.64	15.85	33.25
Standard fertility diet	8.79	7.28	8.37	8.81	12.88
Super-fertility diet	8.95	8.52	7.10	6.46	12.00

4.6.2 The influence of treatment diet on average daily gain and back-fat thickness of Dohne Merino ewes

Table 4.4 presents the influence of the respective treatment diets on the average daily gain (ADG) of the Dohne Merino ewes in the trial, as well as the influence of ewe age on the ADG throughout the trial.

Table 4.4. Descriptive statistics for the influence of treatment diet and age on average daily gain (mean kg ± SE) of mature Dohne Merino ewes that received a flushing diet for a period of six weeks.

Parameter	Mean ADG (kg) ± SE	Range
Treatment diets		
Control	0.045 ± 0.026	-0.134 – 0.189
Lupins	0.088 ± 0.024	-0.100 – 0.208
Standard flushing diet	0.131 ± 0.018	0.043 – 0.262
Super-fertility diet	0.117 ± 0.023	-0.007 – 0.277
Ewe age (years)		
2	0.087 ± 0.017	-0.025 – 0.189
3	0.071 ± 0.025	-0.134 – 0.170
4	0.133 ± 0.030	-0.018 – 0.262
5	0.108 ± 0.038	-0.039 – 0.277
6	0.084 ± 0.030	-0.100 – 0.184

When the non-homogenous start live weights of the ewes were taken into account; no interaction was observed between ewe age and treatment diet (P=0.385); and will thus not be discussed further.

When the live weight of the ewes at the beginning of the trial was taken into account; age did not influence the ADG of ewes throughout the trial (P=0.911; Table 4.5).

Table 4.5. The influence of ewe age on the average daily gain (mean kg ± SE) during the treatment period of six weeks.

Ewe age (years)	Mean ADG (kg) ± SE	Range
2	0,106 ±0.028	0.048 – 0.165
3	0,102 ±0.028	0.045 – 0.159
4	0,084 ±0.031	0.020 – 0.148
5	0,081 ±0.025	0.030 – 0.132
6	0,076 ±0.026	0.023 – 0.129

Feeding of the SFD and SUFD diet resulted in an improved ADG throughout the study period, when compared to the Control and Lupins diets (P=0.006; Table 4.6).

Table 4.6. The influence of treatment diet on the average daily gain (mean gain (kg) ± SE) during the treatment period of six weeks.

Treatment diet	Mean ADG (kg) ± SE	Range
Control	0.015^{b} ± 0.028	-0.042 – 0.072
Lupins	$0.098^{a,b}$ ± 0.24	0.049 – 0.147
Standard flushing diet	0.125^{a} ± 0.024	0.076 – 0.174
Super-fertility diet	0.122^{a} ± 0.024	0.073 – 0.170

^{a,b} Different superscripts in rows denote significant difference (P≤0.05)

The SFD resulted in the highest (P≤0.05) weight gain (0.125 ± 0.024g), followed by the SUFD (0.122 ± 0.024g), when compared to the lupins diet (0.098 ± 0.024g) and the control diet (0.015 ± 0.028g). The ewes in the SFD group gained on average 3.875kg during the trial period of 23 days, compared to SUFD ewes that gained 3.782kg, the ewes on the lupins diet that gained 3.038kg, and the ewes in the control group that gained 0.0465kg.

When only the influence of the lupins diet, and the SFD and SUFD on the ADG of the ewes for the duration of the trial was investigated, no significant influence of diet was observed (P≥0.05; Table 4.6).

Table 4.7 presents the influence of the respective treatment diets on the back-fat thickness of the Dohne Merino ewes in the trial, as well as the influence of ewe age on the back-fat thickness throughout the trial. There was no significant interaction observed between treatment diet and age, for either measurement interval, and thus will not be discussed further.

Table 4.7. Descriptive statistics for the influence of treatment diet and ewe age on the difference in back-fat thickness (mean ± SE) of mature Dohne Merino ewes during the trial period.

Parameter	Mean back-fat thickness (mm) ± SE	Range
Treatment diet		
Control	0.757 ± 0.068	0,622 - 0.893
Sweet lupins	0.814 ± 0,067	0.678 - 0.950
Standard flushing diet	0.878 ± 0,067	0.741 - 1.016
Super-fertility diet	0.831±0,067	0.697 - 0.966
Ewe age		
2	0.316 ± 0.068	0.178 - 0.455
3	0.198 ± 0.081	0.031 - 0.364
4	0.063 ± 0.075	-0.090 - 0.216
5	0.034 ± 0.069	-0.109 - 0.176
6	-0.011 ± 0.078	-0.171 - 0.149

When the non-homogenous back-fat thickness of the ewes at the beginning of the trial (18 September 2019) was taken into account and related to that measured on 30 October 2019, age significantly influenced the back-fat thickness in certain age groups (P=0.004; Table 4.8). Back-fat thickness declined in 5-year old ewes, when compared to the other age groups (-0.011 ± 0.078; P≤0.05). The largest increase in back-fat thickness was observed in 3-year old ewes, when compared to the other age groups (0.316 ± 0.068mm; P≤0.05).

Table 4.8. The influence of ewe age on back-fat thickness (mean ± SE) recorded during the trial.

Ewe age (years)	Mean back-fat (mm) ± SE
2	0.316 ± 0.068
3	0.198 ± 0.081
4	0.063 ± 0.075
5	0.034 ± 0.069
6	-0.011 ± 0.078

[a,b] Different superscripts in rows denote significant difference (P≤0.05)

When the non-homogenous back-fat thickness of the ewes at the start of the trial were taken into account; no significant effect of treatment diet on back-fat thickness was observed (P≥0.665; Table 4.9).

Table 4.9. The influence of treatment diet on the back-fat thickness (mean ± SE) during the treatment period.

Treatment diet	Mean back-fat thickness (mm) ± SE	Range
Control	0,878 ± 0.068	0,741 – 1.016
Lupins	0,831 ± 0.067	0,697 – 0.966
Standard flushing diet	0,814 ± 0.067	0,678 – 0.950
Super-fertility diet	0,757 ± 0.067	0,622 – 0.893

4.6.3 The influence of treatment diet on follicle development

The influence of treatment diet on the percentage contribution of number of follicles counted per treatment group and ewe age is presented in Table 4.10. The number of follicles counted was not influenced by treatment diet (P=0.887; Table 4.10) and ewe age (P=0.223; Table 4.10).

Table 4.10. Descriptive statistics for the influence of treatment diet and ewe age on the percentage contribution of number of follicles tot the total number of follicles counted, as influenced by treatment diet, and ewe age.

Parameter	Percentage contribution to total follicle count (number of follicles)
Treatment diet	
Control	24,62% (81)
Sweet lupins diet	26,44% (87)
Standard flushing diet	24,01% (79)
Super-fertility diet	24,92% (82)
Ewe age (years)	
2	21,88% (72)
3	20,97% (69)
4	22,19% (73)
5	16,72% (55)
6	18,24% (60)

The influence of treatment diet and ewe age on the number of follicles observed, average follicle size, and follicle size range are presented in Table 4.11.

Table 4.11. Descriptive statistics for the influence of treatment diet and ewe age on the numbers of follicles observed, follicle size (mean ± SE), and follicle size range observed in mature Dohne Merino ewes that received a flushing diet for a period of six weeks.

Parameter	Number of follicles	Mean follicle size (mm) ± SE	Range (follicle size in mm)
Treatment			
Control diet	501	4.267 ± 0,088	2,176 - 5.629
Lupins diet	549	4.337 ± 0,080	2,508 - 6.404
Standard flushing diet	508	4.413 ± 0,076	2,780 - 6.108
Super-fertility diet	460	4.262 ± 0.080	2,163 - 5.949
Ewe age			
2	418	4.412^{a} ± 0.101	2,176 - 6.214
3	408	4.412^{a} ± 0.079	2,909 - 5.752
4	504	$4.271^{a, b}$ ± 0.085	2,780 - 6.404
5	330	4.097^{b} ± 0.094	2,163 - 5.591
6	358	4.408^{a} ± 0.092	2,743 - 6.108

^{a,b} Different superscripts in rows denote significant difference (P≤0.05)

Ewe age significantly influenced average follicle size (P=0.007; Table 4.11). Follicle size measured in ewes aged 5 years were smaller, when compared to follicle size measured in two-year-, three-year-, and six-year old ewes (P≤0.002; Table 4.11). Mean follicle size were the largest for 2-year and 3-year old ewes (4.412 ± 0.101 mm), followed by ewes six- and four years of age (4.408 ± 0.092 mm and 4.271 ± 0.085 mm, respectively), the smallest for the 5-year old ewes (4.097 ± 0. 094 mm) (Table 4.11).

There was no significant influence of treatment diet (P=0.330; Table 4.11) on the variation observed in the average follicle size measured for the respective treatment groups. The interaction between treatment diet and ewe age on the average follicle size are presented in Table 4.12.

Table 4.12. Descriptive statistics for the interaction between treatment diet and ewe age on the average follicle size (mean ± SE), and follicle size range observed in mature Dohne Merino ewes that received a flushing diet for a period of six weeks.

Treatment X Age interaction	Mean ± SE	Range
Control diet X		
2 years	4,252[a,b] ± 0.206	2,176-5.629
3 years	4,481[a,b] ± 0.171	3,594-5.322
4 years	4,294[a,b] ± 0.193	3,031-5.164
5 years	4,194[a,b] ± 0.178	2,731-5.549
6 years	4,306[a,b] ± 0.206	2,743-5.461
Sweet lupins diet X		
2 years	4,515[a,b] ± 0.294	2,508-6.214
3 years	4,440[a,b] ± 0.134	2,909-5.198
4 years	4,048[a,b] ± 0.201	2,942-6.400
5 years	4,409[a,b] ± 0.183	2,792-5.910
6 years	4,555[a,b] ± 0.133	3,133-5.443
Standard flushing diet X		
2 years	4,429[a,b] ± 0.220	2,978-5.174
3 years	4,266[a,b] ± 0.148	2,930-5.329
4 years	4,459[a,b] ± 0.140	2,780-5.422
5 years	4,349[a,b] ± 0.140	2,993-4.780
6 years	4,860[a] ± 0.218	3,822-6.108
Super-fertility diet X		
2 years	4,517[a,b] ± 0.130	3,022-5.449
3 years	4,603[a,b] ± 0.195	3,250-5.752
4 years	4,502[a,b] ± 0.155	3,110-5.949
5 years	3,884[b] ± 0.236	2,163-4.901
6 years	4,225[a,b] ± 0.198	3,312-5.259

[a,b] Different superscripts in rows denote significant difference (P≤0.05)

From Table 4.12 it is clear that the biggest variation in mean follicle size observed within a treatment group was for group 3 (SUFD) with a range of 2.163mm–5.949mm. The smallest variation in mean follicle size was observed in the Control group with a total range of 2.176mm–5.629mm. When the average follicle size for all age categories and treatment diets were considered, a significant interaction between treatment diet and ewe age was observed in the SFD for ewes aged 6 years (P=0.036), and for the SUFD for ewes aged 5 years (P=0.036; Table 4.12).

4.6.4 Correlation of ewe live weight with average follicle size

Table 4.13 presents the correlation between average ewe live weight and average follicle size, for the respective treatment diets.

Table 4.13. The correlation between average ewe live weight and average follicle size calculated for ewes that received different flushing diets during the 2019 breeding season.

Treatment diet	Correlation Coefficient
Control diet	0,055
Sweet lupins diet	-0,024
Standard flushing diet	0,209
Super-fertility diet	0,149

A weak negative correlation between average ewe live weight and average follicle size was found for the L diet, whilst weak positive correlations were reported for the control, SFD and SUFD diets.

4.7 Discussion

Although flushing is a common reproduction management tool in the sheep industry in South Africa, the physiological mechanisms underlying the influence of nutrition on follicular development remain unclear (Martin *et al.*, 2004). In South Africa, certain animal feeds companies formulate and sell so-called “super-fertility” diets to sheep producers, with the sales pitch focussing on improved follicular development, that in turn will be realized in an improved weaning success and percentage. There is however, no clinical or production studies to confirm these statements. The purpose of this study was therefore to evaluate the influence of a commercially available “super-fertility” diet (SUFD), with a commercially available standard flushing diet (SFD), and a flushing diet consisting of sweet lupins only (L), in average daily gain (ADG), change in back-fat thickness, and follicular development in sexually mature Dohne Merino ewes aged between 2 and 6 years, and that were maintained under normal production conditions.

When the proximal analysis of the treatment diets was compared, it was observed that the diets differed in terms of percentage crude fat, percentage crude protein, percentage crude fibre, and percentage ash. The L diet was characterized by a higher crude fibre content, when compared to the SFD and SUFD diets (15.85% vs. 8.81% and 6.46%, respectively). Similarly, the L diet was also characterized by a higher percentage crude protein, when compared to the SFD and SUFD diets (5.32% vs. 2.06% and 1.92%, respectively). The SFD was characterized by a higher percentage crude fat, when compared to the L and SUFD diets (8.37% vs. 5.64% and 7.10%, respectively). The percentage ash was the highest for the

SUFD, when compared to the SFD and L diets (8.52% vs. 3.16% and 7.28%, respectively). Regardless of proximal composition, no feed refusals were recorded, thus diet composition did not influence feed intake. This is contradictory to the statement of Rios-Rincón *et al.* (2014), that stated that feed intake is regulated by dietary energy density in ruminants.

When the inclusion of raw materials as per diet formulation is considered, the exact combination and type of protein and energy (i.e. non-structural carbohydrate or lipid in nature) that were included in the SUFD diet cannot be disclosed. Fish oil or sunflower oil are possible ingredients in the SUFD that may increases fertility. Supplementing ewes with fish oil and sunflower oil prior to mating increased the number of medium follicles and size of the ovulatory follicle in fat-tailed Iranian Afshari ewes (Mirzaei-Alamouti *et al.*, 2018). Diets rich in n-6 (safflower oil, canola oil, soy oil, corn oil, sunflower oil) and n-3 (flaxseed oil, canola oil, soybean oil, walnut oil) PUFA have also been found to increase the mean diameter of the ovulatory follicle (Ambrose *et al.*, 2006; Mendoza *et al.*, 2011) and CL (Petit *et al.*, 2002) was higher when dairy cows were fed diets high in n-3 PUFA. The opposite effect was found when dairy cows were fed diets high in n-6 PUFA prior to mating with a lower mean follicle size (Homa and Brown, 1992). Supplementation with n-6 PUFA has not exclusively been detrimental to the follicular development of ruminants seeing that when the n-6 PUFA and n-3 PUFA were compared by Thomas (1997) and found that the number of medium sized follicles was higher when beef cows were supplemented with sources that included n-6 PUFA.

After a correction was made for the non-homogeneous initial weights of the ewes, treatment diet significantly contributed to the variability in ADG of the ewes (P=0.024). Significant differences between treatment diets SFD and the Control (P=0.006), and between SUFD and the Control (P=0.006) persisted to explain the variability in ADG of the ewes. None of the other treatments were significantly different. The SFD contributed to the highest weight gain, bringing an ADG of 0.125g followed by SUFD (0.122g) then L (0.098g) and last C (0.015g). Group 2 ewes (SFD) gained on average 3.875kg over the 23 days of the trial that the ewes were weighed. Group 3 (SUFD) ewes gained 3.782kg in the 23 days, group 1 (L) ewes 3.038kg and the control group 0.0465kg.

This order in which the groups gained weight can be ascribed to the actual fat% (energy levels) of the different flushing diets. Bypass fat facilitates the modification of subcutaneous and intracellular fat deposition in the meat of animals (*Mohsin et al.*, 2019). Fat does not only increase the energy content of diets, but may also increase the daily gain and feed efficiency of animals (Walt *et al.*, 2014). Contradictory to results found by Keane (2019) did the ages of

the ewes not significantly influence the ADG of the ewes. Ewe lambs differ from adult ewes by not responding to flushing (Keane, 2019).
The different treatment diets did not influence back-fat thickness between the dates 18 September 2019 and 9 October 2019 (P=0.945), and between 18 September 2019 and 30 October 2019 (P=0.770). The difference in BT between the different ages of the ewes was indeed significant between 18 September 2019 and 30 October 2019 (P=0.018). The biggest difference in BT according to the ages was for age 3 (0.316mm) following by age 4 (0.198mm), 6 (0.063mm), 2 (0.034) and with a negative value for age 5 (-0.011mm). This findings is contradictory to Milerski (2006) that stated that the age of dam influences the backfat thickness mainly by means of body weight. Keane (2019) also stated that the mature ewes are more likely to respond to flushing rather than the lamb ewes.

The gain in backfat thickness for the different treatment groups can be ascribed to the higher energy levels. Contradictory results found by Ruiz-Nuño *et al.* (2009) where a linear increase of backfat thickness were found (1.42, 2.10, and 2.18 mm) in Pelibuey lambs when they were fed with 14, 16, and 18% of CP in high energy diets, respectively (2.80 Mcal/kg ME). In agreement with Ebrahimi *et al*. (2007) who did not observe any effects of protein level (10.5, 12.5, and 14.5%) on back fatthickness in Mehraban lambs fed finishing diets contained moderated energy level (2.50 Mcal/kg ME).

Studies have attempted to determine whether the energy or protein contents of a flushing diet prior to mating are more important, with some of these that indicated that energy was the most important (Memon *et al.*, 1969; Braden and Mattner 1970; Torell *et al.*, 1972). Fletcher (1981) and Davis *et al.* (1981) however, reported significant effects of protein on ovulation rate. The number of follicle counted in this study was not significantly influenced by the different treatments (P>0.887) or the different ages (P>0.223) of the ewes. These results are in agreement with Haresign (1981) and Munoz Gutierrez (2002) that stated that the number of follicles within the ovary is generally not increased by flushing, per se, but rather flushing increases ovulation rate by preventing atresia of growing follicles. The percentage contribution of follicle counts to the total follicle count was highest for the L treatment group (26.4%) followed by the SUFD treatment group (24.9%), the control group (24.6%) and lowest for the SFD treatment group (24.0%).

There was not a significant difference between the treatment diets that explained the variability of the follicle size means of the ewes (P=0.330). Ewe age significantly (P=0.007) contributed to the variability in average follicle size. Significant differences between the ages 5 and 2 (P=0.002), 5 and 3 (P=0.002), and also 5 and 6 (P=0.004) are seen in order to explain the

variability follicle size means. Mean follicle size were highest for ewes of ages 2 and 3 (4.412mm), followed by age 6 (4.408mm) then 4 (4.271mm) and lastly 5 (4.097mm).

A study by Arthur (1958) also found that two-year-old ewes respond to a flushing program quite differently than do mature ewes, but in his case did the mature ewes respond better in terms of weight gain and reproductive performance. The interaction between the treatment diet and age was significant to explain the variability to in the mean follicle sizes during the trial. One interaction between treatment and age was significant to explain the variability in mean follicle size. This interaction was between group 3 ewes (SUFD) with an age of 5 and group 2 ewes (SFD) with an age of 6 years. The lowest mean in follicle size (3.884mm) was attained for ewes of 5 years that received SUFD and the highest mean in follicle size (4.860mm) was for the ewes that were 6 years and received the SFD (Table 4.12). Looking at this interaction between clearly mature ewes (ages 5 and 6) and knowing that the energy contents of these 2 diets are very similar, the differences in the in the accumulation of mean follicle size for the 2 age groups has to be ascribed to different fat compositions in these feeds. No scientific proof that confirms this statement are available.

The correlation between the weights of the ewes and the mean follicle sizes was very weak for all treatment groups. The correlation between the average ewe live weight and average follicle size represent an estimation of the “static effect” of flushing, whilst the nutritional impact of flushing diet composition on live weight and body condition and consequent follicular development is referred to as the “long-term effect” (Gootwine, 2016).This is contradictory to the many results of studies on the influence of ewe’s LW at mating on subsequent reproductive and productive traits (Adams *et al*., 1997; Bartlewski *et al*., 2011; Gonzalez-Bulnes and Veiga-Lopez, 2005; Gulliver *et al*., 2012b; Huchkowsky *et al*., 2002; Ptacek *et al*., 2014).

A possible reason why the treatment diets did not significantly influence the backfat thickness, number of follicles, and average follicle sizes is that the body condition of the prior to the onset of the trial was too good (i.e. BCS of 3 to respond to the beneficial influence of the extra energy and protein in the respective flushing diets (Torre *et al.*, 1991). This correspond to studies where ewes in good body condition (2.5 to 3.0) did not experience any increase in ovulation rate when fed a flushing diet (Russel *et al*., 1969; Gunn *et al*., 1984). Even though there was an increase in weight over the flushing period, a dynamic effect (Habibizad, Riasi, Kohram, and Rahmani, 2015), the increase in weight and body condition might have not been enough in this present study.

The other potential reason in mind is that the herd prolificacy was already very high (the sheep herd on Mariendahl has been selected for fertility for over 25 years) and was there not a lot of space for improvement. This is contradictory to the conclusion found by Torre *et al.* (1991) that stated that the BCS affects fertility more than the prolificacy of ewes.

The possible reason why there was no correlation between the live weights of the ewes and the mean follicle sizes per treatment groups was very strong homogeneity between ewes.

4.7.1 Conclusions

Treatment diet significantly contributed to the ADG of the ewes, and ewe age significantly contributed to the variation observed in back-fat thickness ewes between 18 September 2019 and 30 October 2019. No differences between treatments or the ages of the ewes to explain the variability of follicle counts per group were found to be significant. The differences in ewe age was significant to explain the variability in mean follicle size per group and the only significant interaction between treatments and ewe ages to explain the variability in mean follicle sizes of the ewes was between the ewes (SUFD) with an age of 5 and the ewes (SFD) with an age of 6 years. There was no correlation between live weight gain in response to the treatment diets and the average follicle size.

Chapter 5: The influence of flushing diet on follicular dynamics, expression of reproductive behaviour, conception success, and lambing success of Dohne Merino (*Ovis aries*) ewes

5.1 Abstract

The study investigated the influence of the composition of different flushing diets on follicular dynamics, conception rate, and lambing performance of semi-intensively managed Dohne Merino ewes. A total of 48 sexually mature Dohne Merino ewes with ages ranging from two to six years, were allocated to four treatment groups, including the control group that received no flushing diet. The different treatments were a flushing diet consisting of sweet lupins only, a commercially available industry-standard flushing diet, and a commercially available "super-fertility" flushing diet. The flushing diets were fed at 250g/day/ewe for 6 weeks, i.e. three weeks prior to the introduction of the intact ram until three weeks after the rams was introduced. Parameters recorded included total volume of ovarian follicles, daily gain in follicle size, ratio of small, medium, large, and ovulatory follicles, conception rate, lambing rate, and lamb birth weight. The treatment diets did not influence any of the parameters recorded. Ewe age significantly influenced the gain in follicle size/day. The largest gain in follicle size/day was for the ewes aged 2 (0.087mm SE ± 0.012) followed by 4 (0.082mm SE ± 0.016), 5 (0.080mm SE ± 0.016), 6 (0.076mm SE ± 0.025) and least gain in follicle size for ewes aged 3 (0.060mm SE ± 0.012).

5.2 Introduction

The seasonality of breeding activity in sheep represents an important constraint in the breeding program of commercial flocks. The 'ram effect' is one of various approaches available to manipulate reproduction during the anoestrous season, with the advantages of being easy to implement and inexpensive and appears to be found acceptable in terms of animal wellbeing by consumers. The ram effect is a well-established management method of using teaser rams to initiate follicular activity, in combination with the feeding of flushing diets, in anovulatory ewes, with the biggest physiological response evident in improved follicular development (Martin *et al*., 1986; Rosa and Bryant, 2002; Ungerfeld *et al*., 2004).

Sheep breeds farmed with in tropical areas are characterized by an aseasonal breeding strategy, and thus have the potential to breed continuously throughout a year (Brown and Jackson 1995; Valencia *et al.*, 2006; Arroyo *et al*., 2007). Godfrey *et al.* (2003), however, reported seasonal differences in the number of lambs born, and suggested that this may be due to the fluctuations in the amount and quality of forage available during the year (Martin *et al*. 2004). Low food availability can result in chronic under-nutrition in some animals, with resulting physiological consequences such as compromised production and reproduction (Lassoued *et al.*, 2009). Short-term nutritional deficiencies might negatively affect reproductive activities in sheep, among the most important the expression of normal oestrus behaviour,

which is a key component to a ram for assessing an ewe's readiness to be mated (García *et al*., 2016).

With the onset a breeding season as determined by photoperiod, an ewe will come into heat every 16-17 days. In South Africa are there generally two mating seasons, i.e. in spring and autumn depending on the mating system approach. In a carefully managed flock, a breeding season will be at the longest two months in duration, allowing an ewe 3.5 - 4 chances of coming into heat. The expression of oestrus behaviour is closely linked with ovulation, with the elevated serum oestrogen concentration prior to ovulation being conditional for oestrus heat behaviour to be expressed (Rolls and Author, 2009).

Ovulation rate is influenced by several factors, of which nutrition is the most important factor determining whether the ewe can support the physiological taxing processes of follicular development, and the consequent mating, conception, parturition, and lactation. Over the years several definitions of the effect of nutrition have been formulated, which include the so-called "static effect", the "dynamic effect" and the "immediate effect". The term "static effect" refers to a higher ovulation rate observed in heavy when compared to lighter ewes, while the "dynamic effect" refers to an increase in ovulation rate as a result of an optimal live weight and body condition in the period (i.e. three weeks) prior to mating (Smith and Stewart, 1990).

An antral follicular wave is defined by the growth of one to three follicles from a pool of small follicles (1-3mm in diameter). When these recruited follicles reach a diameter of larger than 5mm and undergo regression, it is termed an anovulatory wave. In cases where ovulation do occur, the cycle is known as an ovulatory wave (Bartlewski *et al*., 1999; Duggavathi *et al*., 2003). In sheep, the average time between two follicular waves is approximately four to five days. The largest follicles that grow to an ostensibly ovulatory diameter of ≥5mm before regression or ovulation comes forward in an orderly sequence throughout the 17-day interval. Under ideal conditions will three to four follicular waves occur in such an interval.

Some commercial animal feed manufacturing companies in South Africa have so called "super-fertility diets" formulated specifically to increase the reproduction rates for ruminants. Some of these diets are very expensive, and diet specifications is considered confidential. Only similarities between the output of such diets can be drawn and given the cost of such diets is very high expectations for the producer accumulated. The exact physiological mechanisms that are influenced by such diets on reproduction is not clear. The assumptions associated with the feeding of super-fertility diets include improved follicular development,

ovulation, conception, which reflects in an improved lambing rate and weaning success. No scientific research however, has been conducted to verify these assumptions.

There are various studies that report on the influence of nutrition on the reproductive performance of livestock species. In sheep, various studies report on the influence of short-term (i.e. four to six days) supplementation with lupin beans improves ovulation rate, with no effect on live weight or body condition (Knight *et al*., 1975; Lindsay, 1976; Oldham and Lindsay, 1984; Stewart and Oldham, 1986; Downing *et al*., 1995b). This effect has been referred to as the "immediate nutrient effect". According to Smith and Stewart (1990), the different effects of nutrition on ovulation rate forms part of one nutritional continuum, which can be measured at different times relative to the commencement of feeding. The effect of nutrition on reproduction-associated processes may depend on a ewe's "net nutritional status", with the latter being determined by the endogenous and exogenous nutrient sources available to the ewe (Downing and Scaramuzzi, 1991).

Ovulation rate responds to short-term high energy intake (flushing) only if the ewe exhibits a body condition of intermediate range (2.0-3.0) (Wentzel,1986). Wentzel (1986) also found that 48 hours following flushing, a 60% increase in blood glucose concentration occurred. Russel (1978) and Erasmus (1990) postulated that blood glucose levels is a fair indicator of the energy status of the animal. According to Venter and Greyling (1994) the higher energy available as a result from flushing that leads to a higher blood glucose concentration is what is beneficial to the reproductive performance of the ewe. They suggested that the result of flushing is a stimulated pituitary gland that releases more luteinizing hormone which increases the ovulation rate.

Work by Fletcher (1981) and Davis *et al*. (1981) has studied the effects of protein on the ovulation rate of ewes. Fletcher (1981) found, at low levels of dietary energy (4 MJ ME/ewe per day), a response to an increase in dietary protein. Davis *et al*. (1981) came across a similar result where, at moderate energy levels (11.1 MJ ME/ewe per day) but not at low levels (6.25 MJ ME/ewe per day), ewes respond to higher dietary protein. An increased ovulation rate resulted after a high protein supplement were fed to ewes for 32 days compared to the ewes not receiving more protein, with the live weights remaining constant for both groups (Davis *et al*., 1981). Fletcher (1981) and Davis *et al*. (1981) reported a response to an increased dietary protein at a constant level of dietary energy intake.

It has been found that the addition of omega-3 polyunsaturated fatty acids as supplemental feeds in the flushing period increases fertility of sheep (Journal and Kili, 2020). The fatty acids

that has an influence on ruminant reproduction are found to be long chain n-3 and n-6 omega fatty acids that are synthesized by the ruminant through the desaturation and elongation of shorter chain n-3 linolenic acid (ALA, 18:3n-3) and n-6 linoleic acid (LA, C18:2n-6). Long-chain n-3 fatty acids (20 carbons or more) can be found in fishmeal and fish oil and are also suitable to be fed to ruminants (Ashes *et al*., 1992). The short-chain n-6 LA can be found in various raw materials such grains, soybean, safflower, and sunflower. In industry, certain supplement are formulated to include as much as 10% of total lipids as n-6 LA (Gulliver et al., 2012b). These supplements of n-3 and n-6 fatty acids are expensive, and uncertainty arises whether the increase in production will justify the input costs.

Although very conservative information around the actual specs of the commercially available diets are available, it is hypothesized that the improved reproduction of ewes fed a super-fertility diet may be a result of a combination of low concentration protein and high-quality fat in the form of n-3- and n-6 PUFA. No clinical data, however, are available to support or refute this hypothesis. One of the main motivations of the current study is thus investigate the influence of flushing diet composition on follicular development, and ultimately lambing success, and to determine whether the higher feed costs of such fertility diets are justified by an improve lambing percentage.

Ovarian ultrasonography is a useful tool in tracking the progression of antral follicular development, and presence of pre-ovulatory follicles, as well as the formation of the corpus luteum (Schrick *et al*., 1993; Gonzalez-Bulnes *et al*., 2000; Duggavathi *et al*., 2003a, b; Vi˜noles *et al*., 2004). It is estimated that it takes more than six months for ovarian follicles to be recruited and grow, from the primary follicle to the preovulatory stage. The time it requires for the recruited primordial follicles to reach a size of 0.2 mm in diameter (i.e. the pre-antral stage) have also been estimated to be approximately 130 days (Cahill and Mauleon, 1980). For the follicles to reach a size of 0.5mm in diameter will require a full 24 to 35 days, and for the follicles to be considered to be in an antral stage where they are 2.2mm in size an additional five days of growth are required. The pre-ovulatory size (i.e. a minimum of 4 mm in diameter) is typically attained about four days later (Turnbull *et al*., 1977). For this normal pattern of follicular recruitment and subsequent development to be realized and to allow a ewe the ability to perform according to her genetic breeding potential, it becomes even more imperative that the nutritional demands of the ewe is properly understood, so that informed nutritional decisions can be made to ensure that the flock's reproduction efficiency is optimized, which in turn will contribute to ensuring food security. Seen against the background of the estimated human population for 2050 (i.e. ± 10 billion), if the feeding of expensive flushing diets (e.g. "super-fertility" diets) do indeed realize in optimal lamb production, this in

turn will potentially alleviate the pressure on natural resources by potentially decreasing the water and carbon and footprint of sheep production.

The purpose of this study was therefore to compare a commercially available “super-fertility” diet, a commercially available industry-standard flushing diet, and a flushing diet consisting of lupins only (the latter which some producers feed to their ewes prior to the mating season), in terms of the effect on total follicle volume, gain in follicle size/day, mean small, medium, large and ovulatory follicles, conception success, lambing success and lamb birth weight of sexually mature Dohne Merino ewes.

5.3 Materials and Methods

Ethical approval was obtained from the Animal Care and Use Ethics Committee of the University of Stellenbosch (ACU Reference #: ACU-2019-6629).

5.3.1 Experimental location

The study was conducted between 18 September and 31 October 2019 on the Mariendahl Experimental Farm of Stellenbosch University, which is located about 14 km outside Stellenbosch in the Western Cape (latitude:-33.843947; longitude: 18.817218). Stellenbosch lies on 128m above sea level, and the area is characterized by a warm, temperate climate. The climate in the region is classified as Csb by the Köppen-Geiger system. The temperature here averages 16.4 °C | 61.5 °F. The annual rainfall, of which most is received during the winter season, is 802 mm.

5.3.2 Experimental animals and husbandry

Forty-eight Dohne Merino ewes, aged between two and six years of age, were used in the study. The live weight of the ewes ranged between 58kg and 95kg, and the average body condition score ranged from 2.5 to 3 (Suiter, 1994).

The ewes originated from one resource flock and were managed as a single mating group throughout the trial. This adhered to the management practice on the farm, where a mating group consist of 50 ewes allocated to one ram. Before the commencement of the trial the ewes were maintained in one large camp and were visually and physically isolated from the breeding rams.

The teaser ram was introduced 13 days prior to the introduction of the intact ram, as part of the behavioural stimulus of the teaser ram effect, used in combination with a flushing diet to synchronize oestrus in the ewes (Gootwine, 2016).

The experimental animals were monitored daily for general wellbeing. Any signs of abnormal behaviour, respiratory abnormality, limping animals, animals lacking energy, enthusiasm or appetite was immediately reported.

5.3.3 Experimental design and treatments

The treatment diets consisted of lupins (treatment 1; L), a commercially available flushing diet (treatment 2; SFD), a commercially available super-fertility diet (treatment 3; SUFD), and a control diet (no flushing diet provided; C). The experimental animals were allocated to the respective treatment groups to ensure that all age groups were represented in the treatment groups, and identified with ear-tags that differed in terms of colour to allow for the easy separation of the ewes for weighing, feeding, and scanning purposes.

The composition of the respective treatment diets is presented in Table 5.1.

Table 5.1. The nutritional composition of the treatment diets fed to Dohne Merino ewes prior to and during the 2019 breeding season.

Diet component	Standard flushing diet	Super embryo diet	Lupins diet
Moisture (%)	11.5	11.47	10.0
Protein (%)	19.44	14.43	37.0
Fat (%)	3.32	4.76	8.9
Fibre (%)	6.24	11.76	10.7
Ash (%)	3.58	8.29	3.0
Starch (%)	32.45	21.67	2.1
NPN Protein (%)	31.9	8.17	0
UDP (%RP)	27.0	29.18	14.0
Urea (%)	1.88	0.29	0
Ca (%)	0.94	1.26	0.3
P (%)	0.48	0.48	0.45
ME (MJ/kg)	10.6	9.8	12.8

The experimental diets were fed to the ewes for six weeks, i.e. for a period of three weeks before the introduction of the intact ram, until three weeks after the intact ram was introduced. Each ewe received 250g/ewe/day, and feeding was carried out in the morning, after completion of blood collection and ultrasound scanning. Any feed refusals were recorded.

The teaser ram was introduced to the experimental flock on 25 September 2019, one week after the start of the feeding of the experimental diets. The teaser ram was maintained with the ewes for a period of 13 days, after which the intact teaser ram was replaced with a ram.

The intact ram was maintained with the ewes for a period of 30 days, after which the intact ram was removed and added to the breeding ram flock maintained on the same farm. The respective rams were maintained with the ewes and were only separated from the ewes when the latter had to be fed and scanned.

Pregnancy diagnosis was performed by an experienced veterinarian technician from Overberg Animal Hospital, 60 days after the removal of the intact ram (García *et al*., 1993). Based on the ultrasound findings, ewes were then separated into flocks of ewes carrying single foetuses, and twins/triplets to allow for the nutritional managements of the ewes according to the number of foetuses they carried. Table 5.2 presents the timeline of specific actions carried out during the trial.

Table 5.2. Timeline of the trial to indicate the intervals at which data recording were carried out.

Date	Action
2019/09/18	SUFD treatment starts, all animal weighing starts, scan back-fat thickness all animals
2019/09/19	L and SFD treatment start
2019/09/25	Teaser ram in/ Follicular scanning protocol begins for SUFD and Control groups
2019/09/26	Follicular scanning protocol begins for L and SFD groups
2019/10/08	Intact ram in/ Teaser ram removed
2019/10/09	Scan back-fat thickness all animals
2019/10/11	2nd last day of weighing (no weighing 12/10- 17/10)
2019/10/18	Weigh all animal one last time
2019/10/30	Last day of flushing fed to SUFD group, scan back-fat thickness all animals.
2019/10/31	Last day of flushing diet fed to L and SFD groups
2019/11/08	Intact ram out
2020/01/07	Pregnancy scan (60 days after ram out)

5.3.4 Detection of oestrus behaviour

Prior to the introduction of the teaser, ewes were physically and visually isolated for a minimum period of six weeks from the breeding rams on the farm. Signs of oestrus in the ewe are much less pronounced than in the cow or doe and can usually not be detected unless a ram is present. A vasectomised ram was used as a teaser ram, and introduced to the ewes on 25 September 2019 to stimulate oestrus, and to allow for detection of oestrus ewes. On 8 October 2019 the teaser ram was removed from the ewes, and an intact ram was introduced. A marking medium comprising of butter and ochre was applied on the chest of the intact ram, which facilitated the identification of ewes that were jumped by the ram. Signs of oestrus in the ewes was recorded daily from when the teaser ram was introduced.

For more detail on the classification of the different oestrus behaviours expressed, please refer to Chapter 3.

5.3.5 Ultrasound scanning

Ewes were scanned daily by the same operator, using transrectal ultrasonography to assess follicular development on the ovaries. Transrectal ultrasonographic examinations were performed using a Mindray DP-30 veterinary ultrasound scanner and a 75L50EAV transducer (7.5 MHz linear array probe).

Scanning commenced seven days after the nutritional trial started, and continued for 16 days (Toosi *et al*., 2009). Settings (near-field, far-field and overall gain) was optimised prior to the beginning of the trail and maintained throughout the study. The number and diameter of all follicles visible on both ovaries were recorded daily. Examinations were performed with the ewe in standing position by external intra-rectal manipulation of the transducer.

Analysis of follicle data required the classification of follicles into the size classes of small, medium, large, and ovulatory. For more detail on the follicle classification, please refer to Chapter 3.

5.3.6 Lambing

Ewes lambed during the February-March 2020 lambing season. The birth weight of each lamb was recorded on the day it was born.

Lambing percentage (LP) is calculated by the following formula:

LP = (Number of lambs born alive/Number of ewes exposed to ram) x 100.

5.3.7 Statistical analyses

Statistical analyses were performed using Microsoft Excel Stat 2020 with modules XLSTAT-Premium. An ANOVA was performed to determine the influence of ewe age and treatment diet on the total follicle volume. An ANOVA was performed using the slope of the average follicle size to determine the average daily gain in follicle size over time. To accommodate the difference in follicle size at the beginning of the ultrasound period, an ANCOVA was performed using the slope of the average follicle size, with the intercept of the slope as a co-variant.

An ANOVA was carried out to determine the influence of treatment diet and ewe age on the number of small, medium, large and ovulatory follicles.

A Chi-square test was carried out on the number of lambs born to determine if treatment diet influenced the number of lambs born. An ANOVA analysis was also performed to determine whether treatment diet and/or ewe age had an influence on lamb birth weight.

5.4 Results

5.4.1 Total follicle volume

Analyses on the total follicle volume were performed to determine if the total follicular volume produced by the ewes was influences by the treatment diets and/or the ages of the ewes (Table 5.3).

Table 5.2: Descriptive statistics for the influence of treatment diet and ewe age on the sum of the follicle size (sum ±SE) of mature Dohne Merino ewes that received a flushing diet for a period of six weeks.

Parameter	Follicle count	Follicle size sum ± SE	Range
Treatment			
Control diet	501	26.862 ± 1,733	4,352 - 63.794
Sweet lupins diet	549	27.632 ± 1,533	2,792 - 67.653
Standard flushing diet	508	28.367 ± 1,648	3,274 - 65.069
Super-fertility diet	460	23.996 ± 1,420	3,250 - 54.268
Ewe Age			
2	418	25.133 ± 1.740	3,274 - 59.193
3	408	25.840 ± 1.663	3,250 - 57.710
4	504	30.027 ± 1.847	5,727 - 65.069
5	330	25.780 ± 1.909	2,792 - 57.852
6	358	26.791 ± 1.692	5,443 - 67.653

Age (P=0.109; Table 5.3) and Treatment diets (P=0.133; Table 5.3) was not significant in order to explain the variability in the sum of the follicle sizes. The interaction between the ewe age and the treatment diets was also not significant (P=0.060).

5.4.2 Gain in follicle size per day

The increase in follicle size per day was analyzed in order to determine if the treatment diets and/or age had an influence on the gain in follicle size per day (Table 5.4).

Table 5.3: Descriptive statistics for the influence of treatment diet and ewe age on the gain in mean follicle size/day (gain/day in mean follicle size ±SE) of mature Dohne Merino ewes that received a flushing diet for a period of six weeks

Parameter	Mean ± SE	Range
Treatment diets		
Control diet	0.096 ± 0.015	0.003 – 0.174
Sweet lupins diet	0.054 ± 0.009	0.011 – 0.106
Standard flushing diet	0.076 ± 0.018	-0.015 – 0.218
Super-fertility diet	0.080 ± 0.013	-0.012 – 0.012
Ewe age (years)		
2	0.087 ± 0.012	0.024 – 0.142
3	0.060 ± 0.012	-0.008 – 0.132
4	0.082 ± 0.016	0.023 – 0.174
5	0.080 ± 0.016	-0.015 – 0.116
6	0.076 ± 0.025	-0.012 – 0.218

When the non-homogenous initial follicle sizes of the ewes were taken into account, the different treatment diets (P=0.196; Table 5-4) did not significantly explain the variability of gain in follicle size/day within each individual ewe. The ewe ages (P=0.041; Table 5.4) was significant to explain the variability in gain in follicle size/day. The largest gain in follicle size/day for the ages of the ewes was for age 2 (0.087mm) followed by 4 (0.082mm), 5 (0.080mm), 6 (0.076mm) and least gain in follicle size for age 3 (0.060mm).

Table 5.5 portrays the differences between gain in follicle size/day accumulated by different ages of the ewes.

Table 5.4: Analysis of the differences between gain in follicle size/day accumulated by different ages of the ewes with a confidence interval of 95% (gain in follicle size/day)

Age	Mean follicle ± SE	Range
2	0.079 ^{a,b} ± 0.004	0.070 - 0.087
3	0.078 ^{a,b} ± 0.004	0.070 - 0.086
4	0.072 ^{a,b} ± 0.004	0.064 - 0.081
5	0.066 ^{b} ± 0.005	0.056 - 0.075
6	0.086 ^{a} ± 0.004	0.077 - 0.095

^{a,b} Different superscripts in rows denote significant difference (P≤0.05)

Differences between ages 5 and 6 (P=0.004) was significant in order to explain the variability in gain in follicle size/day within the ewes once the initial sizes of the follicles were taken into account.

The interaction between treatment diets and ages of the ewes was significant to explain the variability in gain in follicle size over time. Table 5.6 portrays the interactions between treatment diets and the ages of the ewe.

Table 5.5: Interaction between age and treatment on the increase in follicle size/day(mm)

Age*Treatment	Mean±SE	Range
2*Control	0,073 ^{a,b} ± 0.008	0,057 - 0.090
2*Lupins	0,082 ^{a,b} ± 0.009	0,063 - 0.100
2*SFD	0,078 ^{a,b} ± 0.009	0,060 - 0.097
2*SUFD	0,082 ^{a,b} ± 0.007	0,067 - 0.097
3*Control	0,071 ^{a,b} ± 0.008	0,056 - 0.087
3*Lupins	0,081 ^{a,b} ±0.007	0,066 - 0.096
3*SFD	0,068 ^{b} ± 0.008	0,052 - 0.083
3*SUFD	0,093 ^{a,b} ± 0.009	0,074 - 0.111
4*Control	0,075 ^{a,b} ± 0.010	0,055 - 0.095
4*Lupins	0,054 ^{b} ± 0.009	0,035 - 0.073
4*SFD	0,080 ^{a,b} ± 0.007	0,065 - 0.095
4*SUFD	0,079 ^{a,b} ± 0.007	0,064 - 0.095
5*Control	0,070 ^{a,b} ± 0.009	0,051 - 0.089
5*Lupins	0,074 ^{a,b} ± 0.009	0,056 - 0.093
5*SFD	0,070 ^{a,b} ± 0.009	0,051 - 0.089
5*SUFD	0,049 ^{b} ± 0.009	0,030 - 0.068
6*Control	0,071 ^{a,b} ± 0.009	0,057 - 0.090
6*Lupins	0,084 ^{a,b} ± 0.008	0,063 - 0.100
6*SFD	0,121 ^{a} ± 0.009	0,060 - 0.097
6*SUFD	0,067 ^{b} ± 0.009	0,067 - 0.097

^{a,b} Different superscripts in rows denote significant difference (P≤0.05)

From Table 5.6 was there four significant interactions between treatments and age in order to explain the variability in follicular size gain/day. The following include those interactions: age 6 in group 2 (SFD)*age 3 in group 2 (SFD), age 6 in group 2 (SFD)*age 5 in group 3 (SUFD), age 6 in group 2 (SFD)*age 2 in group 1(L) and lastly age 6 in group 2 (SFD)*age 6 in group 3 (SUFD).

5.4.3 Follicle categories

For follicles to reach an antral stage where they are 2.2mm in diameter or larger, 30 to 40 days of growth after the initial size of 0.2mm is necessary. The preovulatory size (at least 4mm in diameter) is typically attained about 4 days later (Turnbull *et al*., 1977). When these recruited follicles reach a diameter of larger than 5mm and undergo regression, it is termed an anovulatory wave. In cases where ovulation occurs, the cycle is known as an ovulatory wave (Bartlewski *et al*., 1999; Duggavathi *et al*., 2003). The follicles in the trial was categorized in groups according to sizes (small, medium, large and ovulatory) to determine if the different treatment diets and/or ages of the ewes influenced the follicular dynamics at different development phases.

5.4.3.1 Small follicles

The follicles that had a diameter of <3mm was categorized as small follicles. Table 5.7 portrays the descriptive statistics of the small follicles per treatment group and ewe age.

Table 5.6: Descriptive statistics for the influence of treatment diet and ewe age on the mean of the follicle size (Sum ±SE) for the small follicles of mature Dohne Merino ewes that received a flushing diet for a period of six weeks.

Parameter	Follicle count	Mean small follicle size ± SE	Range
Treatment			
Control	81	2,406 ± 0.267	1,625 – 2.992
Lupins	78	2,490 ± 0.272	1,354 – 2.992
Standard flushing diet	78	2,370 ± 0.272	1,354 – 2.992
Super-fertility diet	69	2,404 ± 0.289	1,360 – 2.991
Ewe Age			
2	70	2,479 ± 0.287	1,625 – 2.991
3	51	2,401 ± 0.333	1,354 – 2.991
4	72	2,359 ± 0.283	1,354 – 2.991
5	56	2,413 ± 0.319	1,605 – 2.992
6	57	2,436 ± 0.316	1,360 – 2.992

Treatments (P>0.480) and ewe age (P>0.709) was not significant in order to explain the variability of the means of the small follicles.

5.4.3.2 Medium follicles

The follicles that consisted of diameter of between 3mm and 5mm were categorized as medium follicles. Table 5.8 portrays the descriptive statistics of the medium follicles per treatment group and ewe age.

Table 5.7: Descriptive statistics for the influence of treatment diet and ewe age on the mean of the follicle size (Sum ±SE) for the medium follicles of mature Dohne Merino ewes that received a flushing diet for a period of six weeks

Parameter	Follicle count	Mean Medium follicle size ± SE	Range
Treatment			
Control	254	4,095 ± 0.153	3,008 – 5.00
Lupins	306	4,050 ± 0.139	3,004 – 4.996
Standard flushing diet	250	4,089 ± 0.154	3,005 – 5.000
Super-fertility diet	242	4,093 ± 0.156	3,016 – 5.000
Ewe Age			
2	191	4,074 ± 0.176	3,008 – 4.996
3	215	4,065 ± 0.166	3,005 – 5.000
4	275	4,059 ± 0.147	3,004 – 4.996
5	184	4,105 ± 0.179	3,004 – 5.000
6	187	4,109 ± 0.178	3,016 – 5.000

Treatments (P>0.788) and ewe age (P>0.710) was not significant in order to explain the variability of the means of the medium follicles.

5.4.3.3 Large follicles

The follicles that consisted of sizes between 6mm and 5mm were categorized as large follicles. Table 5.9 portrays the descriptive statistics of the large follicles per treatment group and ewe age.

Table 5.8: Descriptive statistics for the influence of treatment diet and ewe age on the mean of the follicle size (Sum ±SE) for the large follicles of mature Dohne Merino ewes that received a flushing diet for a period of six weeks

Parameter	Follicle count	Mean Large follicle size ± SE	Range
Treatment			
Control	122	5,430 ± 0.219	5,008 – 5.989
Lupins	120	5,498 ± 0.221	5,014 – 5.995
Standard flushing diet	140	5,473 ± 0.205	5,008 – 5.982
Super-fertility diet	110	5,476 ± 0.230	5,020 – 5.973
Ewe Age			
2	116	5,439 ± 0.225	5,020 – 5.973
3	112	5,486 ± 0.228	5,008 – 5.984
4	116	5,456 ± 0.225	5,008 – 5.989
5	70	5,460 ± 0.287	5,014 – 5.973
6	78	5,519 ± 0.272	5,028 – 5.995

Treatments (P>0.295) and ewe age (P>0.731) was not significant in order to explain the variability of the means of the large follicles.

5.4.3.4 Ovulatory follicles

An analysis on the ovulatory follicles was done. The ovulary follicles was identified as follicles greater than 6mm in diameter. The analyses on ovulatory follicles could only have been done from the 16th day that the ewes have been in the trial seeing that only from there all groups had ovulatory follicles present and visible. Table 5.10 describes the results found on the ovulatory follicles.

Table 5.9: Descriptive statistics for the influence of age and treatment diet on mean ovulatory follicle size over time within mature Dohne Merino ewes that received a flushing diet for a period of six weeks

Parameter	Follicle count	Mean ovulatory follicle size (mm) ± SE	Range
Treatment			
Control	44	6,271 ± 0.023	6.022 - 6.500
Lupins	45	6,202 ± 0.022	6.004 - 6.500
Standard flushing diet	40	6,237 ± 0.026	6.016 - 6.500
Super-fertility diet	39	6,237 ± 0.022	6.016 - 6.500
Ewe age			
2	41	6,231 ± 0.024	6,004 - 6.500
3	30	6,216 ± 0.032	6,016 - 6.464
4	41	6,227 ± 0.023	6,022 - 6.500
5	20	6,164 ± 0.023	6,004 - 6.500
6	36	6,299 ± 0.023	6,016 - 6.500

Ewe age (P=0.864) and treatment diets (P=0.229) was not significant to explain the variability of the mean ovulatory follicle sizes of the ewes.

Figure 5.1 portrays the follicle count/size category/treatment group.

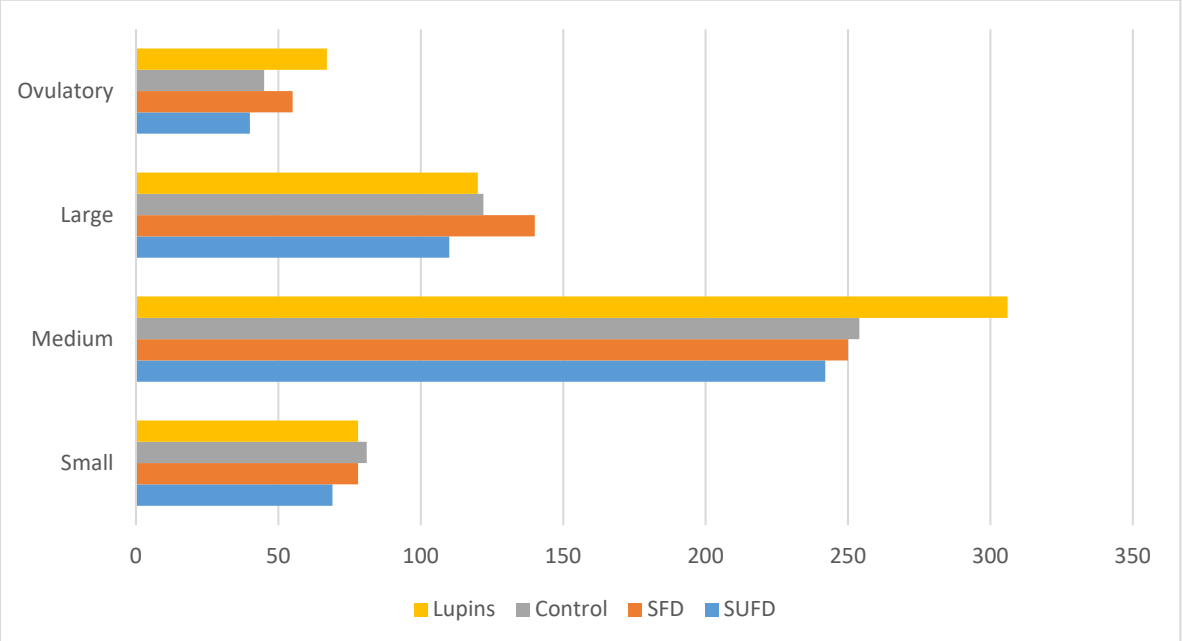

Figure 5.1: Frequency of follicles per size category within the ewes of the respective treatment groups.

5.4.4 Behaviour

Given the size of the trial group was the behavioural data recorded by in terms of mountings. A marking medium comprising of butter and ochre was applied on the chest of the intact ram, which facilitated the identification of ewes that were jumped by the ram, assuming that when they have this greasy stain on their hinds that they were in oestrus (Hawken and Martin, 2012). Table 5.11 indicates the count of mountings observed per day that the ram was present in the trial.

Table 5.11: Contingency table of the ewes mounted by the ram during the time the ram was present.

Mounted by Ram\ Treatment	Super fertility diet	Standard fertility diet	Control	Lupins	Total
Ram in day 2	5	1	2	3	11
Ram in day 3	4	7	6	4	21
Ram in day 4	0	3	2	2	7
Ram in day 5	2	1	1	1	5
Ram in day 10	1	0	1	0	2
Ram in day 11	0	0	0	2	2
Total	12	12	12	12	48

On day 3 was 21 mounting observed in the herd. The closest count of mountings to day 3 during the time the ram was present in the trial was on day 2, where 11 mountings were observed. During the first 5 days of the ram present in the trial were 44 mountings observed. It is estimated that the average ram mates more than 45 times per week in a large flock and re-mates with ewes three to four times (Fraser and Broom, 1998). On days 10 and 11 of the ram present in the herd was the last 4 ewes mounted.

5.4.5 Conception rate

Table 5.12 indicates the number of ewes that conceived per treatment group.

Table 5.10: Results for the amount of lambs observed via ultrasound scan/treatment group by an experienced veterinarian technician from Overberg Animal Hospital

Treatment \ #lammers	0	1	2	3	Total ewes scanned	Total lambs observed
Super fertility diet	0	4	8	0	12	20
Standard fertility diet	0	5	7	0	12	19
Control	1	5	6	0	12	17
Lupins	0	5	7	0	12	19
Total ewes scanned	1	19	28	0	48	75
Total lambs observed	0	19	56	0	75	

From Table 5.8. the conception percentage for the experimental group of ewes for 2019 was 156.3%.

5.4.6 Lambs

Table 5.13 indicates the amount of lambs born per treatment group.

Table 5.11: Results for the amount of lambs born per treatment group.

Treatment \ #lammers	0	1	2	3	Total ewes lambed	Total lambs born
Super fertility diet	0	3	8	1	12	22
Standard fertility diet	0	4	8	0	12	20
Control	1	4	7	0	12	18
Lupins	0	5	7	0	12	19
Total ewes lambed	1	16	30	1	48	79
Total lambs	0	16	60	3	79	

The lambing percentage for the experimental group of ewes for 2019 was 166.7%. Lambing percentage is calculated by the following formula: LP = (Number of lambs born alive/Number of ewes exposed to ram) x 100. The treatment diets (P= 0.675) and the ages of the ewes (P= 0.899) was not significant in order to explain the variability in lambs born.

Group 3 (SUFD) was the only treatment group that produced triplets. Group 3 was also the treatment group that produced the highest yield in live lambs born (22), followed by group 2, SFD (20), group 1, L (19) and lastly the Control group (18). From Table 5.13 is it also evident that the Control group was the only group with an ewe that did not produce a lamb.

5.4.7 Lamb Total Birth Weight

The influence of treatment diet and ewe age on the number of lambs born, average lamb birth weight and lamb birth weight range are presented in Table 5.14

Table 5.12: Descriptive statistics for the influence of treatment diet and ewe age on average birth weight (mean BW ± SE) of the lambs of Dohne Merino ewes that received a flushing diet for a period of six weeks

Parameter	Lamb count	Mean lamb BW (mm) ± SE	Range
Treatment			
Control	18	7,158 ± 0.668	5,790 - 8.527
Lupins	19	6,950 ± 0.668	5,581 - 8.319
Standard flushing diet	22	7,692 ± 0.668	6,323 - 9.060
Super-fertility diet	20	8,325 ± 0.668	6,956 - 9.694
Ewe age			
2	16	6,800 ± 0.698	5,371 - 8.229
3	21	8,509 ± 0.698	7,080 - 9.938
4	15	7,978 ± 0.771	6,398 - 9.558
5	13	7,175 ± 0.818	5,499 - 8.851
6	14	7,100 ± 0.771	5,520 - 8.680

Ewe age and treatment diet did not contribute to the differences in total birth weights of the lambs (P=0.485 and P=0.446, respectively). There is also no significant interaction between the ages and treatment diets to explain the variation of the total lamb birth weights (P=0.675).

Despite the observation that one ewe of the Control diet did not produce a lamb, this did not result in the Control having the lowest total lamb birth weight.

5.5 Discussion

Age (P=0.109) and treatment diet (P=0.133) did not influence the variation observed for the sum of the follicle size. The interaction between the ewe age and the treatment diets was also not significant (P=0.06). Ewes aged four years achieved the largest sum in follicle size (30.027mm), followed by ewes aged six years (26.791mm), ewes aged three years (25.840mm), ewes aged five years (25.780mm), with the lowest sum in follicle size reported for ewes aged two years (25.133mm). This difference in order can be ascribed to a more frequent recruitment of antral follicles. The higher frequency in small follicles adds up to a higher total follicular volume compared to a ewe with larger follicles, but smaller total follicular volume(Ślósarz *et al*., 2003)

When the non-homogenous initial follicle sizes of the ewes were taken into account, the different treatment diets (P=0.196) did not significantly explain the variability of gain in follicle size/day within each individual ewe. The ages (P=0.041) differed significantly in order to explain the variability in gain in follicle size/day. The largest gain in follicle size/day for the ewe age was for ewe age 2 (0.087mm) followed by 4 (0.082mm), 5 (0.080mm), 6 (0.076mm) and least gain in follicle size for ewe age 3 (0.060mm). This result is in agreement with study by Arthur (1958) who found that two-year-old ewes respond to a flushing program quite differently than do mature ewes.

Treatments (P=0.480) and ewe age (P=0.709) was not significant in order to explain the variability of the means of the small follicles (<3mm). This is an important finding considering that the fluctuation in the number of 2mm and 3mm follicles is used to describe the recruitment of follicles at wave emergence and their inhibition by the dominant follicle (Gil, 2003).

Treatments (P=0.788) and ewe age (P=0.710) was not significant in order to explain the variability of the means of the medium follicles. The majority of the follicles observed over the course of the trial was medium follicles (3mm- 5mm). This is contradictory to the results found by Gil (2003) where the majority of follicles observed was 2mm.

The accuracy of identifying follicles with a size greater than 4mm is high via ultrasound scanning of ewes in standing position (Gil, 2003). Treatments (P=0.295) and ewe age (P=0.731) was not significant in order to explain the variability of the means of the large follicles (5mm-6mm).

Follicles that where greater than 6mm were assumed to be ovulatory follicles. When follicles reach a diameter of larger than 5mm and they undergo regression, it is termed an anovulatory wave. In cases where ovulation occurs, the cycle is known as an ovulatory wave (Bartlewski *et al*., 1999; Duggavathi *et al*., 2003). During the trial only from day 16 onwards did all groups have ovulatory follicles present. Ewe age (P=0.864) and treatment diets (P=0.229) was not significant to explain the variability of the mean ovulatory follicle sizes of the ewes. The interaction between the ewe ages and the treatment diets was also not significant (P=0.785). This results is contradictory to the results of a study on the induction of oestrus, ovulation and gestation in ewe lambs and mature ewes out of season (Martinez *et al*., 2015) where the mature ewes had significantly higher ovulation rates than the ewe lambs. A possible reason for this contradictory result may be that the genetic potential for fertility of the ewes on Mariendahl is very good, seeing that the herd have been selected for fertility for over 25 years.

According to Carolina Viñoles (2003) is inconsistent descriptions between investigators found specially for follicles between 2mm and 3mm via ultrasound scanning of ewes in standing position in order to identify and measure follicle diameter. A study on the effect of lupin supplementation on fertility of Ripollesa ewes also found no significant influences by flushing (Torre *et al*., 1991). Habibizad *et al*., (2015) stated that short-term flushing with high energy or high protein diets affected the number of different size follicles and double ovulation rate of cyclic ewes, but the exact mechanism controlling the effects of nutritional supplementation on folliculogenesis and the growth of dominant follicles is unclear. In his study he found increased follicular development in terms of number of large follicles and number of ovulatory follicles, but no significant differences between the two energy supplements. This is in agreement to the present study where no significant differences between treatments diets was observed to explain the variability in the follicle size sum, gain in follicle size/day, mean small, medium, large and ovulatory follicles.

The control group had very high frequency mountings on day 3. On day 3 was 21 mounting observed in the herd. The closest count of mountings to day 3 during the time the ram was present in the trial was on day 2, where 11 mountings were observed. During the first 5 days of the ram present in the trial were 44 mountings observed. It is estimated that the average ram mates more than 45 times per week in a large flock and re-mates with ewes three to four times (Fraser and Broom, 1998). On days 10 and 11 of the ram present in the herd was the last 4 ewes mounted. This is a clear indication of the ram effect and that the oestrus cycles of the ewes that were very well synched (Lezama *et al*., 2003).

The number of ewes mounted soon after the introduction of the ram gives an estimation of the sexual partner preference of the ram. The ram sexual partner preference is according to Roselli *et al* (2011) stimulated by oestrus behaviour. Short-term dry matter deficiency during mating season induces changes in foraging behaviour of sheep ewes and decreases ram-seeking behavior (J. García et al., 2016). In the present study is it clear that the ram's preference for the ewes was homogeneously spread between treatment groups.

The treatment diet (P= 0.675) and ewe age (P= 0.899) was not significant in order to explain the variability in lambs born. The lambing percentage for the experimental group of ewes was very high for 2020 (166.7%). The SUFD treatment group was the only group that produced triplets, and the highest yield in live lambs born (22 lambs). The SFD group produced 20 lambs, followed by the L treatment group that produced 19 lambs, and the Control group that produced 18 lambs. The control group was the only group with an ewe that did not lamb.

An observation was made on the yield in mean follicle sizes (Chapter 4), ovulatory follicle sizes and live lambs born. Treatment diets was not significant in order to explain the variability in mean follicle sizes, ovulatory follicle sizes or the live lambs born, the yield produced is noteworthy. From highest to lowest yield per treatment diet is the order the same for mean follicle size and ovulatory follicle size, with the SUFD group having the highest yield, followed by the SFD, the control, with L having the lowest yield. A different descending order in yield is seen when looking at the live lambs born. Here the SUFD group produced the most lambs, followed by the SFD group, then the L group, with the and then group 1 (L) and lastly with the least lambs produced by the Control group. The Control group was also the only group with an ewe that did not lamb.

Lamb birth weight is the outcome measure related to maternal characteristics and individual energy intake of the ewe during any single pregnancy (Gardner *et al*., 2007). No significant differences between the ages (P=0.485) of the ewes or treatment diets (P=0.446) in order to explain the differences in total birth weights of the lambs. SUFD still seems to have produced the highest yield, followed by SFD then the control and then L. The control (group 4) produced more lamb live weight than the lupins, even though the lupins (group 1) gave birth to more lambs (group 1 gave birth to 19 lambs, group 4 gave birth to 18 lambs).

No significant differences between the ewe ages (P=0.485) of the ewes in order to explain the differences in total birth weights of the lambs. The youngest ages and the oldest ages, (2 and 6) had the lowest produce of lamb/kg (total live body weight). The ewe age that had the highest produce of lamb/kg (total body weight) where 3 followed by 4 and 5. This result is almost exactly in agreement with the results found by Ptáček *et al*. (2017) where the differences among particular age groups was not significant but the number of live lambs born were lowest for ewes of ages 2 and 6 years.

5.5.1 Conclusions

Ewe age and treatments were not significantly different in order to explain the variability in the sum of the follicle sizes. Treatments was not significantly different to explain the variability of gain in follicle size/day within each individual ewe. Ewe ages was indeed significantly different in order to explain the variability in gain in follicle size/day. Treatments and ewe age was not significantly different in order to explain the variability of the means of the small follicles (<3mm). Treatments and ewe age was not significantly different in order to explain the variability of the means of the medium follicles. Treatments and ewe age (P>0.731) was not significantly different in order to explain the variability of the means of the large follicles (5mm-

6mm). Treatments and ewe age was not significantly different to explain the variability of the mean ovulatory follicle sizes of the ewes. The treatments and the ages of the ewes was not significantly different in order to explain the variability of live lambs born. No significant differences between treatments or the ewe ages in order to explain the differences in total birth weights of the lambs.

Chapter 6: General Conclusion and recommendations

In South Africa, livestock producers and especially sheep producers, are faced with various challenges such as global warming and high input costs, and thus are under increased pressure to farm as cost-efficiently as possible. The pressure to produce enough lamb and mutton to meet the demand by 2020, necessitate that farmers need to be able to manage their flocks efficiently to allow for the production of safe food.

The management of the reproductive potential of sheep flocks is crucial to ensure the viability and sustainability of sheep production systems, whether wool-, lamb- or mutton production is the focus. Flush feeding and the use of a teaser ram are two proven management tools that can be used to prepare a ram and ewe for the physiological taxing processes associated with reproduction. Flush feeding involves the use of a diet that will provide the ewe with protein and energy in excess to what she requires for daily maintenance, and therefore this supplemental energy and protein can be partitioned by the ewe toward the initiation of follicular development in the ovaries, as well as prepare her body for the physiology stress of reproduction. The teaser ram is used, through the action of pheromones, to stimulate the ewe to initiate follicular development in the ovaries, as influenced by the increase in the associated reproductive hormones upon exposure to the teaser ram. A combination of these two practices should allow an ewe to produce according to her genetic potential.

For the ewe to respond effectively, it is essential that the flushing diet is formulated properly to ensure that the ewe will be able to mobilise the extra energy and protein supplemented through the flushing diet. Flushing diets are normally more expensive than maintenance diets, due to the higher protein and energy being included. The raw materials that serve as source for the protein and energy in the flushing diet, contribute largely to the costs of a flushing diet. In South Africa, certain feed companies formulate flushing diets that they market as so-called “super-fertility” diets, with the assumption that feeding of such diets will be ultimately be reflected in an improved lambing percentage and thus in return-on-investment (ROI). There are, however, no studies to support of refute these assumptions. The purpose of this study was therefore to compare a commercially available super-fertility diet with a commercially available standard flushing diet, and also a sweet lupins diet, in its ability to improve the reproductive response and ultimately the lambing performance of Dohne Merino ewes.

The purpose of the study was therefore to compare different flushing diets in terms of the influence on the lambing performance of sexually mature Dohne Merino ewes. Reproductive management of the ewes in this study involved the use of a teaser ram that was introduced to

the ewes for a period of 13 days, after which this ram was replaced with an intact ram. The intact ram was maintained with the ewes in a breeding flock for a period of 30 days, which allowed sufficient opportunity for all ewes to be mated. Pregnancy diagnosis was carried out 60 days after the intact ram was separated from the ewes, and ewes were managed according to the number of foetuses that were identified to ensure that the nutritional needs of the ewes were met. Lamb birth weights were recorded, and the lambing performance calculated. The treatment diets (including a control group) were fed to sexually mature Dohne Merino ewes, that ranged from two to six years in age, and that were maintained in a semi-intensive production system. Ewe parameters recorded included live weight, calculated average daily gain (ADG), back-fat thickness (BT), and number of lambs born. Ovary-related data recorded included number of follicles, average follicle size, total follicle volume, ratio of small, medium, large, and ovulatory follicles. Follicular data were consequently related with ewe conception rate, and lambing percentage. The L diet can be classified as a high-fibre, high-protein, and low-fat diet. The SFD and SUFD can both be classified as high-fibre, high-fat and low-protein diets.

The influence of flushing diet composition on live weight, back-fat thickness, and follicle development

The ADG of the ewes was significantly influenced by flushing diet composition, which can be considered as a dynamic effect of flushing (Coop, 1966). The SFD-fed ewes gained 0.125g/day, followed by the SUFD-fed ewes (0.122g/day), the L-fed ewes (0.098g/day), with the control group that gained the least during the study (0.015g/day). Back-fat thickness was measured at the beginning and end of the study period, and BT was significantly influenced by ewe age. The most pronounced increase in BT was observed in ewes three years old (0.316mm), followed by ewes aged four years (0.198mm), ewes aged 6 years (0.063mm), ewes aged 2 years (0.034), with the ewes aged five years that lost BT throughout the study (-0.011mm).

Ewe age significantly contributed to the variation in average follicle size, where average follicle size was the largest for ewes aged two and three years (4.412mm), followed by ewes aged six years (4.408mm), ewes aged four years (4.271mm), with ewes aged five years producing follicles with the smallest average size (4.097mm). The interaction between treatment and age was significant for ewes that were 5 years of age and that received the SUFD, and ewes six years of age that received the SFD. No correlation was observed between ewe live weight and average follicle size.

The influence of flushing diet on the follicular dynamics, expression of oestrous behaviour, conception rate, and lambing percentage

The treatment diets did not influence any of the parameters recorded. Ewe age significantly influenced the gain in follicle size/day. The largest gain in follicle size/day was reported for ewes aged two years (0.087mm), followed by ewes aged four years (0.082mm), ewes aged five years (0.080mm), ewes aged six years (0.076mm), with ewes aged three years showing the least gain in follicle size/day (0.060mm).

When considering the influence of the respective flushing diets on the reproduction behaviour of the ram and ewes, it would appear that ewes that received the SUFD and SFD diets were mated in the first half of the mating period, more than the L-fed ewes and ewes in the Control group.

Several studies report on the effect of supplementary feeding and/or the direct or indirect effect of poor rangeland conditions on ewe reproductive performance (Rhind *et al*., 1989; Molle *et al*., 1997; O'Callaghan and Boland, 1999; Branca *et al*., 2000; Santra *et al*., 2002; Chaturvedi *et al*., 2003). Ocak *et al*. (2006) showed that a short-term (2 weeks) increase in level of protein supplementation during the post-mating period improved the reproductive performance of ewes maintained on rangeland. Other studies found that ovulation rate responds to short-term high energy intake (i.e. the purpose of the flushing diet) only if the ewe exhibits an intermediate body condition (i.e. 2.0-3.0) (Wentzel,1986).

When the raw materials included in the respective treatment diets are considered, the exact combination and type of protein and energy (i.e. non-structural carbohydrate or lipid in nature) sources included in the SUFD or SFD cannot be disclosed. It is hypothesized that fish oil or sunflower oil are potential energy sources that may have been included in the SUFD that with the aim of improving follicular development and ultimately lambing percentage. may increases fertility. Supplementing ewes with fish oil and sunflower oil prior to mating increased the number of medium follicles and size of the ovulatory follicles in fat-tailed Iranian Afshari ewes (Mirzaei-alamouti et al., 2018). Diets rich in n-6 PUFA (safflower oil, canola oil, soy oil, corn oil, sunflower oil) and n-3 PUFA (flaxseed oil, canola oil, soybean oil, walnut oil) have also been found to increase the average diameter of the ovulatory follicle (Ambrose *et al.*, 2006; Mendoza *et al.*, 2011).

In summary, the SFD and SUFD diets had the most beneficial influence on the ADG of the ewes, and thus allowed the ewes from these treatment groups to maintain their body condition throughout the mating period. Backfat thickness, however, and even though it is considered

more objective than body condition scoring, was not sensitive enough to indicate a loss in body condition. The BT of ewes aged 5 years, regardless of diet fed, declined throughout the study period, and the impact of this decline was evident in the smaller average follicle size reported for this age group. Number of follicles counted per ewe was not influenced by either the treatment diet of ewe age.

When the proximate analysis results of the diets are compared, it is evident that even though the specifications are almost similar, that a difference in the costs of the two diets (i.e. SFD = R2500/ton; SUFD = R5253/ton) imply that more expensive protein and energy sources were used in the formulation of the latter.

To determine against the background of the findings of this study and to determine the financial implications of feeding the more expensive SFD and SUFD dets, a very basic return-on-investment (ROI) calculation was performed. The calculations assume that the lambs born during the study will be sold at weaning, and that the current price for live weaned lambs are used (ABSA-red meat prices on 25/9/2020 = R37.78/kg live weight; source Agriorbit, 2020). No other production and overhead costs were considered in the calculations, therefore only the L-, SFD- and SUFD diets will be compared in terms of ROI.

Information pertaining to calculation:

Feed price per ton:

- L diet = R 3 000/ton (i.e. R3.00/kg)
- SFD = R 2 500/ton (I.e. R2.50/kg)
- SUFD = R 5 253/ton (i.e. R5.25/kg)

Total feed consumption per ewe for duration of the study:

- 0.25kg/day X 42 days = 10.5 kg

Total feed costs per treatment diet:

- L diet = 10.5kg X R3.00 = R31.50 per ewe. For group = 31.50X12 = R378.00
- SFD = 10.5kg X R2.50= R26.25 per ewe. For group = 26.25X12 = R 315.00.
- SUFD = 10.5kg X R5.25= R55.13 per ewe. For group = 55.13X12 = R 661.56.

Income from selling of weaned lambs (average weight at weaning = 25kg) per treatment group:

- L treatment group (19 lambs) = 19 X 25 X R37.78 = R 17 945.50.
- SFD treatment group (20 lambs) = 20 X 25 X R37.78 = R 18 890.00.
- SUFD treatment group (22 lambs) = 22 X 25 X R37.78 = R 20 779.00.

Calculation of return on investment (ROI): ROI = Net Profit / Total Investment

- L treatment group = 17 945.50/378.00 = R47.47.
- SFD treatment group = 18 890/315.00 = R59.97.
- SUFD treatment group = 20 779/661.56 = R31.41.

From the ROI calculations, it is evident that the feeding of the "super-fertility" diet in this study did not outperform the L- and SFD diets, respectively. The use of SUFD thus is aimed at a niche group of producers, i.e. producers that have access to superior genetics and use of assisted reproductive technologies (ARTs) such as artificial insemination and embryo production. The costs of a SUFD and use of ARTs will potentially be offset by the improved rate of growth and production by the offspring of the animals of higher genetic merit. It is thus not suitable for use in sheep breeding systems where ARTs are not used to optimise the prolificacy of a flock.

Recommendations for future studies

Future studies need to investigate the potential of the SUFD and SFD to improve the reproductive performance and ultimately number of lambs/ewe, in ewes that have a body condition score of 2.5 or less. The composition of the flushing diets, especially in terms of type protein and/or energy source, on the follicular dynamics and consequent reproductive performance of ewes, needs to be investigated. Of special interest here are two aspects, i.e. the inclusion of PUFAs and the minerals and vitamins included in the flushing diet. When the proximal analysis of the SFD and SUFD is considered, this diet was characterized by a much higher percentage Ash values than the L diet (7.28% and 8.52% vs. 3.16%).

When the influence of the flushing diet on the reproductive behaviour is consider, and when this is related to the influence of the SFD and SUFD diets, it would appear that the SFD-fed and SUFD-fed ewes were mated more during the first half of the mating period. Future studies is suggested to investigate this complex interaction between type of energy and protein included in the ewe's flushing diet, and how this influence the hypothalamus-pituitary-gonad axis with the ewe's exposure to the teaser and intact rams, respectively. This may shed more light on how and to what extent the supplemented energy and protein in the flushing diet is portioned towards reproduction-related processes in the ewe.

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